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DEDICATION

I dedicate this thesis to my beloved family, whose unwavering support and encouragement have been my guiding light throughout this journey. To my parents, for their endless sacrifices and belief in my potential; to my siblings, for their constant inspiration and motivation; and to my friends, for their understanding and companionship during the challenging times.

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Thank you all for being a part of this journey.

Wissem

DEDICATION

This thesis is dedicated to my family, whose unwavering support has been a constant source of strength throughout my academic endeavors. Your encouragement and understanding have made this journey possible.

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Unconditional love….

Ines

TABLE OF CONTENTS:

ACKNOWLEDGMENTS
DEDICATIONS
LIST OF FIGURES
LIST OF TABLES
LIST OF ABBREVIATIONS
ABSTRACT
RESUME
ملخص
INTRODUCTION..01
THE THEORETICAL PART
CHAPTER I. GENERAL INFORMATION ON *SPIRULINA*
I.1 History 03
I.2 Definition 04
I.3 Classification 05
I.4 Morphology and Physiology 05
I.5 Habitat and Geographic 06
I.6 Life cycle and Reproduction 06
I.7 Cultivation and production of *Spirulina* 07
I.7.1 Cultivation of *Spirulina* 07
I.7.1.1 Culture Parameters 07
I.7.1.1.1 Temperature 07
I.7.1.1.2 Hydrogen potential (pH) 07
I.7.1.1.3 Salinity The growth of *Spirulina* 07
I.7.1.1.4 Light *Spirulina* 08
I.7.1.1.5 Oxygen and agitation 08
I.7.2 Production of *Spirulina* 08
I.7.2.1 Artisanal production 08
I.7.2.2 Semi-industrial production 09
I.7.2.3 Industrial production 09
I.8. Main applications of *Spirulina*..10
I.8.1 In human food 10
I.8.2 In cosmetics 10

I.8.3 In medicine 12
I.8.4 In animal feed 12
I.8.5 In agri-food 12

CHAPTER II. BIOCHEMICAL COMPOSITION OF *SPIRULINA*.

II.1 Composition of *Spirulina platensis* 13
II.1.1 Protein 13
II.1.2 Lipids 14
II.1.2.1 Total lipids 14
II.1.2.2 Fatty acids 14
II.1.3 Carbohydrates 14
II.1.4 Vitamins 15
II.1.4.1 Beta-carotene (provitamin A) 15
II.1.4.2 Tocopherols (vitamin E) 15
II.1.4.3 Group B vitamins 15
II.1.4.4 Vitamin B12 16
II.1.5 Minerals 16
II.1.6 Nucleic acids 17
II.1.7 Pigments 17
II.1.7.1 Chlorophyll 17
II.1.7.2 Phycocyanin 18
II.1.7.3 Beta-carotene 18
II.1.8 Enzymes 18

CHAPTERIII.BIOLOGICAL ACTIVITIES OF *SPIRULINA*

III.1 Antioxidant activity 19
III.1.1 Oxidative stress 19
III.1.1.1 Free radicals 19
III.1.1.2 Antioxidants and defense systems 19
III.1.1.2.1 Endogenous antioxidants 19
III.1.1.2.2 Exogenous antioxidants 20
III.1.2 Antioxidant potential of S. Platensis 20
III.2 Other activities 20
III.2.1 Antibacterial activity 20
III.2.2 Anti-inflammatory activity 20
III.2.3 Antiviral activity 21

III.2.4 Anti-cancer activity 21
III.2.5 Anti-hypercholesterolemic activity 22
III.2.6 The therapeutic aspect 22

THE PRACTICAL PART

CHAPTER I. MATERIALS AND METHODS

I. Materials 23
I.1. Biological material 23
I.2. Non-biological material and equipment 23
I.2.1 Microscopic observation of *Spirulina* 23
I.2.2 Preparation of extracts 24
I.2.3 Determination of pigments contents (Chlorophyll a, b and Carotenoids) 24
I.2.4 Extraction of phycocyanin 24
I.2.5 Evaluation of antioxidant activity 25
I.2.6 Determination of the antibacterial activity 25
II.Methods 25
II.1. Microscopic observation of *Spirulina* 25
II.1.1Wet mount examination 25
II.1.2 Gram staining 26
II.2. Preparation of extracts 26
II.2.1 Calculation of dry extract yields 27
II.2.3 Determination of pigments contents (Chlorophyll a, b and Carotenoids) 29
II.2.4 Extraction of phycocyanin 30
II.2.4.1 Solvent Extraction 30
II.2.4.2 Extraction by maceration in glycerol 32
III. Study of Biological activities 33
III.1. Evaluation of antioxidant activity 33
DPPH free radical inhibition test 34
III.2. Determination of the antibacterial activity 37
III.2.1Preparation of bacterial cultures 38
III.2.2 Antibacterial activity by disc diffusion method 39

CHAPTER II. RESULTS AND DISCUSSION

II.1 Microscopic observation of *Spirulina* 41
II.2 Yield of extracts obtained 42
II.3 Content of chlorophyll a, b and carotenoids 43

II.4 Extraction of phycocyanin 44
II.5 Biological activities 45
II.5.1 Antioxidant activity 45
II.5.2 Antibacterial activity 48
CONCLUSION 52
BIBLIOGRAPHIC REFERENCES
APPENDICES
APPENDIX A

LIST OF FIGURES

Figure.1	*Spirulina* in the Chadian market **(Koru, 2012).**	03
Figure.2	*Spirulina* under microscope **(J.P. Jourdan, 2013).**	04
Figure.3	Various microscopic aspects of *Spirulina* **(Goulamabasse, 2018).**	05
Figure.4	Geographic distribution of *Spirulina* **(Fox, 1999).**	06
Figure.5	Biological reproduction cycle of *Spirulina* **(Charpy *et al*., 2008).**	07
Figure.6	Various artisanal cultures of *Spirulina* **(Jourdan,2018).**	09
Figure.7	Improved basins in Hawaii **(Théodore,2017).**	10
Figure.8	Average chemical composition of *Spirulina* **(Goulambasse, 2018).**	13
Figure.9	Positioning of *Spirulina* in relation to other foods in terms of protein levels (%) **(Vrenna *et al*., 2021).**	14
Figure.10	*Spirulina* powder.	23
Figure.11	Ethanol and hydro-ethanol extraction protocol from *Spirulina* powder by maceration.	28
Figure.12	Protocol for determination of pigments contents (Chlorophyll a, b and Carotenoids).	30
Figure.13	Protocol for extraction of phycocyanin by solvent.	31
Figure.14	Extraction process of phycocyanin by maceration in glycerol.	32
Figure.15	Protocol for extraction of phycocyanin by maceration in glycerol.	33
Figure.16	Protocol for evaluation of antioxidant activity.	36
Figure.17	The antibacterial activity of *Spirulina* extracts using the disc diffusion method.	40
Figure.18	Microscopic observation of *S. platensis* at 10X and 40X magnification.	41
Figure.19	Microscopic observation of *Spirulina* with 100X objective lens after Gram staining.	41
Figure.20	Content of chlorophyll a, b and carotenoids in (mg/g).	43
Figure.21	Concentrations and degrees of purity of phycocyanin obtained by two methods of extraction.	44
Figure.22	Percentage of DPPH inhibition as a function on different concentrations of 2 types of *Spirulina platensis* extracts.	46
Figure.23	IC50 of different *Spirulina* extracts and ascorbic acid.	47

LIST OF TABLES

Table.1	Taxonomy of the species *Spirulina platensis.*	05
Table.2	Various *Spirulina* productions and their characteristics (**Charpy *et al., 2008***).	08
Table.3	*Spirulina* benchmark on skin care products **(Ragusa *et al, 2021*).**	11
Table.4	Vitamin content **(Falquet, 1996).**	15
Table.5	Mineral content **(Falquet, 1996).**	16
Table.6	Nucleic acid content **(Borwitzka, 1988).**	17
Table.7	Pigments in *Spirulina* powder **(Ali *and Saleh, 2012*).**	17
Table.8	Tools, apparatus, solutions and reagents used in the characterization of *Spirulina* strain.	23
Table.9	Tools, apparatus and solutions used in preparation of extracts.	24
Table.10	Tools, apparatus and solutions used to determination of pigments contents.	24
Table.11	Tools, apparatus and solutions used to extraction of phycocyanin.	24
Table.12	Tools, apparatus and solutions used in the evaluation of antioxidant activity.	25
Table.13	Tools, apparatus and solutions used in the determination of the antibacterial activity.	25
Table.14	Reference bacteria strains used in the study	37
Table.15	Extraction yield from *Spirulina platensis* strain.	42
Table.16	Diameters of inhibition zones of ethanolic extract of *Spirulina* on eight bacterial strains.	49
Table.17	Diameters of inhibition zones of hydro-ethanolic extract of *Spirulina* on eight bacterial strains.	49

LIST OF ABBREVIATIONS

µm: Micrometer (micron)
A.platensis*: *Arthrospira platensis
A0: the absorption of the witness.
AA: amino acid
Ai: sample absorption.
AMR : antimicrobial resistance
ATCC: American Type Culture Collection
Ca: Calcium
Car: Carotenoid
Chll: Chlorophyll
CMV: human cytomegalovirus
COX-2: Cyclooxygenase-2
CRBT: the biotechnology research center
DMSO: Dimethyl Sulfoxide
DNA: Deoxyribonucleic Acid
DPPH: 2,2-diphenyl-1-picrylhydrazyl
E. coli: *Escherichia coli*
FDA: Food and Drug Administration
Hb: Hemoglobin
HIV-l: Human Immunodeficiency Virus Type 1
HSV: Herpes Simplex Virus
I%: inhibition ratio
IC50: Half Maximal Inhibitory Concentration
IL-1β : Interleukin 1 Beta
IL-6 : Interleukin 6
K: Potassium
M0: Quantity of plant powder used for the extraction expressed in g
M1: Quantity of extract recovered expressed in g
Mg: Magnesium
MH: Mueller-Hinton
Na: Sodium
Na_2CO_3: Sodium Carbonate
$NaHCO_3$: Sodium Bicarbonate
NGOs: Non-Governmental Organizations
O_2: Oxygen
pH: Hydrogen potential
R %: Extract yield expressed in g/100g of dry matter
RNA: Ribonucleic Acid
RNS: reactive nitrogen species
ROD: reactive oxygen derivatives

ROS: reactive oxygen species
S.aureus: *Staphylococcus aureus*
S.platensis**:** *Spirulina platensis*
SOD: superoxide dimutase
TNF α : Tumor Necrosis Factor Alpha
UN: United Nations
UNESCO: United Nations Educational, Scientific and Cultural Organization
Vit C: Vitamin C
WHO: World Health Organization
Zn: Zinc

ABSTRACT

Spirulina, a filamentous cyanobacterium, is a source of antioxidants and has antibacterial potential. Quantitative analysis of certain bioactive compounds revealed the presence of chlorophyll a (18.31 mg/g); chlorophyll b and carotenoids were present at concentrations of 9.94 mg/g and 1.91 mg/g, respectively. Phycocyanin extraction was performed using a solvent and also by maceration in glycerol. The latter method yielded a higher concentration of phycocyanin (0.26 mg/ml) and higher purity (0.91) compared to the solvent extraction method (concentration: 0.21 mg/ml and purity: 0.83).

The antioxidant activity was evaluated using the DPPH free radical inhibition test. The IC50 values of the ethanolic and hydro-ethanolic extracts were 0.69 mg/ml and 0.65 mg/ml, respectively.

Antibacterial activity tests showed that *Spirulina* extracts exhibited inhibitory effects against various tested bacterial strains, with the ethanolic extract showing superior activity.

Keywords: *Spirulina* p*latensis*, chlorophyll, phycocyanin, antioxidant activity, antibacterial activity.

RESUME

La spiruline, une cyanobactérie filamenteuse, source d'antioxydants et à potentiel antibactérien. L'analyse quantitative de certains composés bioactifs a révélé la présence de la chlorophylle a (18,31 mg/g) ; la chlorophylle b et les caroténoïdes étaient présents à des concentrations de 9,94 mg/g et 1,91 mg/g, respectivement. L'extraction de phycocyanine a été réalisée en utilisant un solvant et par macération dans le glycérol. Cette dernière a permis d'obtenir une concentration en phycocyanine (0,26 mg/ml) et une pureté plus élevées (0,91) par rapport à la méthode d'extraction par solvant (concentration : 0,21 mg/ml et pureté : 0,83).
L'activité antioxydante a été évaluée en utilisant le test d'inhibition des radicaux libres DPPH. Les valeurs IC50 des extraits éthanoliques et hydro-éthanolique étaient respectivement de 0,69 mg/ml et de 0,65 mg/ml.
Les tests d'activité antibactérienne ont montré que les extraits de *Spirulina* ont présenté des effets inhibiteurs contre diverses souches bactériennes testées, l'extrait éthanolique manifestant une activité supérieure.
Mots-clés : *Spirulina platensis*, chlorophylle, phycocyanine, activité antioxydante, activité antibactérienne.

ملخص

السبيرولينا، وهي بكتيريا زرقاء خيطية، تُعد مصدرًا لمضادات الأكسدة ولها إمكانات مضادة للبكتيريا. كشفت التحليلات الكمية لبعض المركبات النشطة بيولوجيًا عن وجود الكلوروفيل أ (18.31 ملغم/غ)؛ وكان الكلوروفيل ب والكاروتينات موجودين بتركيزات بلغت 9.94 ملغم/غ و 1.91 ملغم/غ على التوالي. تم استخراج الفيكوسيانين باستخدام مذيب وعن طريق النقع في الجلسرين. هذه الأخيرة مكنت من الحصول على تركيز أعلى من الفيكوسيانين (0.26 ملغم/مل) ونقاء أعلى (0.91) مقارنة بطريقة الاستخلاص بالمذيب (تركيز: 0.21 ملغم/مل ونقاء: 0.83).
تم تقييم النشاط المضاد للأكسدة باستخدام اختبار تثبيط الجذور الحرة DPPH. كانت قيم IC50 للمستخلصات الإيثانولية والمائية-الإيثانولية 0.69 ملغم/مل و 0.65 ملغم/مل على التوالي.
أظهرت اختبارات النشاط المضاد للبكتيريا أن مستخلصات السبيرولينا أظهرت تأثيرات مثبطة ضد العديد من السلالات البكتيرية المختبرة، حيث أظهر المستخلص الإيثانولي نشاطًا أعلى.

الكلمات المفتاحية: *Spirulina platensis*، الكلوروفيل، الفيكوسيانين، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا.

INTRODUCTION

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to medicines, making people sicker and increasing the risk of disease spread, illness and death. AMR is largely driven by the misuse and overuse of antimicrobials. **(World Health Organization, 2024).** This situation highlights the urgent need to develop new therapeutic approaches to combat these pathogens.

One microscopic blue algae appeared with the first living organisms about 3.5 billion years ago and is considered the most complete natural food. This is the Cyanobacterium *Arthrospira platensis*, better known as *Spirulina* **(Cruchot., 2008).** It has attracted the attention of researchers for many years, and its potential benefits have been experimentally proven *in vitro* and *in vivo* for the treatment of certain pathologies and in the prevention of hypercholesterolemia, certain inflammatory diseases, allergies, cancer, drug-induced toxicity, viral infections, cardiovascular diseases, and diabetes, among others **(Khan *et al., 2005;* Kulshreshtha *et al., 2008;* Karkos *et al., 2008*).**

Sipirulina naturally grows in alkaline waters of certain lakes in tropical and subtropical areas **(Fox, 1999).** It is grown across the world in three distinct methods (traditional, semiindustrial, and industrial), where it may develop naturally because the right circumstances (pH, salinity, and temperature) are present for its growth. Five thousand tons are produced there annually. 90% of it is utilized as human food, while 10% is used as animal feed for things like fish and fowl **(Ahsan *et al., 2008*).**

Spirulina is an exceptionally potent source of antioxidants. In fact, one ton of fruits contains the same amount of antioxidants as just one kilogram of *Spirulina* **(Manet, 2016).** *Spirulina* is rich in a variety of antioxidant compounds, including phycocyanin, β-carotene, vitamins A, C, and E, the enzyme superoxide dismutase (SOD), gamma-linolenic acid, selenium, and methionine. Furthermore, studies have shown that the activities of most of the body's own (endogenous) antioxidant systems are increased after the administration of *Spirulina* **(Moor *et al*., 2020).**

It is in this context that our interest in these micro-algae was born, through this work which provides a synthetic vision of its potential, primarily through the assigned objectives in this context:

- Determination of pigments content in this material.
- Extraction of phycocyanin by two methods.
- Evaluation of the antioxidant activity of *Spirulina*
- Determination of the antibacterial activity of *Spirulina* against these reference strains: *Escherichia coli ATCC 25922, Salmonella enteritidis 13076, Salmonella typhi 94028,*

Staphylococcus aureus ATCC 25923, Klebsiella pneumonia 700603, *Bacillus subtilis 6633, Bacillus cereus 10876, and Pseudomonas aeruginosa 27853.*

Our work is structured in two parts:

The first part is dedicated to a bibliographic synthesis. This section contains three chapters:

- ❖ The first chapter will provide general information about *Spirulina*.
- ❖ The second chapter presents the biochemical compositions of *Spirulina*.
- ❖ The third title chapter describes the various biological activities of *Spirulina*.

The second part is dedicated to the experimental section. It addresses the materials and the different methods used for the completion of the work. This part gathers all the results, which will then be followed by a discussion.

Finally, a general conclusion on the entire study will be provided, complemented by potential future perspectives.

THE THEORETICAL PART

CHAPTER I.
GENERAL INFORMATION ON *SPIRULINA*

I.1 History

Cyanobacteria were detected over 3.5 billion years ago and produced the first breathable oxygen for organisms **(Sguera., 2008),** *Spirulina* was previously thought to be a blue-green algae (cyanophycée), but it was actually classified as a cyanobacteria by Wittrock and Nordsted under the name *Spirulina* in 1844 **(Bensehaila., 2016).**

For centuries, the Kanembous people in Chad and the ancient Aztecs of the Texcoco Valley in Mexico have been consuming and harvesting *Spirulina*. The first recording of this was by Cortes, a conquistador, in 1521 who wrote about eating sun-dried *Spirulina* pancakes. *Spirulina* was rediscovered in Chad in 1930 by a pharmacist from the colonial troops, and Brandilly, an anthropologist and filmmaker, wrote an article titled "Since ages, an African tribe in Chad has been exploiting the food of the year 2000." However, Western scientists only became interested in *Spirulina* much later **(Adiba *et al., 2011*).**

In the twentieth century, Christopher Hill became associated with the history of the use of *Spirulina*. Hill, is American, discovered that *Spirulina* provided an incredible solution to the nutritional problems of our time and was easy to "cultivate" since it grows on its own with just a little water and sunlight. *Spirulina* is one of the most amazing foods on earth **(Lafri., 2018).**

Several dozen scientific studies on *Spirulina* have been conducted by researchers around the world since the 1980s, and we still have much to learn about the beneficial effects of daily consumption of *Spirulina* **(Girardin *et al., 2011*).**

Figure 01: *Spirulina* in the Chadian market **(Koru, 2012)**

I.2 Definition

Spirulina is a photosynthetic, filamentous and multicellular blue-green microalgae that grows in a wide range of fresh, marine and brackish water. It thrives in a highly alkaline environment with a pH of 10-12 **(Marrez, *et al.,2014*).** Its scientific name is *Arthrospira platensis* **(Henrikson,1989).** A procaryotic organism that shares with plants the ability to perform photosynthesis. From mineral compounds, water, and captured light energy through their chlorophyll, they transform carbon dioxide and release oxygen. **(Ahounou ,2018).**

Spirulina is considered to be a highly nutritious food source because of its high protein content (70%) and its digestibility, particularly due to its high content of phycocyanin, which makes it turn green during photosynthesis **(Fox, 1994).**

It is therefore natural for *Spirulina* to be used as a food supplement, and sometimes as a complete meal. Its richness in nutrients has attracted the attention of researchers. Currently, its uses are numerous. It is no longer just a question of nutritional value. It has entered the pharmaceutical, cosmetic and food industries **(Scheldeman *et al., 1999*).**

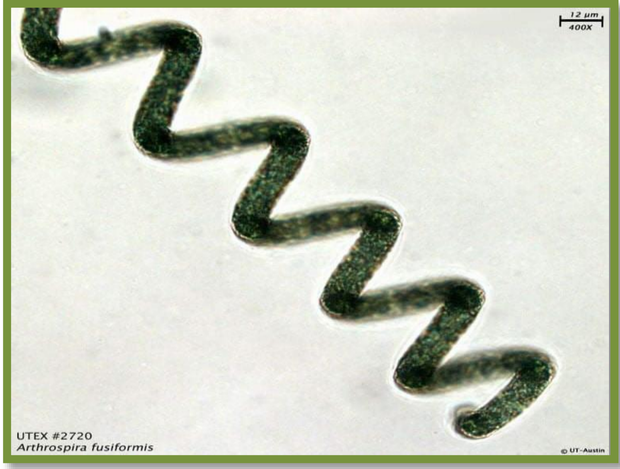

Figure 02: *Spirulina* under microscope **(J.P. Jourdan, 2013)**

I.3 Classification

Spirulina is a cyanobacterium (formerly referred to as "blue algae" or cyanophyceae). It belongs to the domain of bacteria it has a cell wall similar to that of gram-negative bacteria. Cyanobacteria make up the bulk of bacteria capable of photosynthesis with oxygen production. **(Charpy *et al., 2018*)**

According to **Fox (1999),** the systematic position is as follows:

Table 1: Taxonomy of the species *Spirulina platensis*

Kingdom	*Monera*
Sub-kingdom	*Prokaryote*
Phylum	*Cyanophyta*
Class	*Cyanophyceae*
Order	*Nostocalles*
Family	*Oscillatoriaceae*
Genus	*Arthrospira*
Species	*Arthrospira platensis*

I.4 Morphology and Physiology

Spirulina has an average length of 250 µm when it has seven spirals. It is composed of mobile, non-branched filaments (10-12 µm in diameter) wound in spirals, typically in six or seven spirals, resembling a small coil spring, hence the name "*Spirulina*" **(Geitler, 1932, Jarisoa, 2005).**

More specifically, *Spirulina* is made up of transparent cells stacked end to end to form a filament or trichome. The winding of the trichome on itself occurs in a clockwise direction when viewed from above the spiral. Environmental factors such as temperature are believed to have an influence on the orientation of the helix **(Muhling *et al., 2003*, Jarisoa, 2005).**

Spirulina filaments are motile, often moving by twisting movements at speeds of more than 5µ per second, with motility serving to protect it from excessive sunlight exposure **(Fox, 1999).**

It typically takes on various shapes including "spiral", "undulating", and "straight" **(Vicente, 2012).** This unique form is directly related to the ecological conditions encountered in its habitat **(Charpy *et al., 2008*).** (Fig.3)

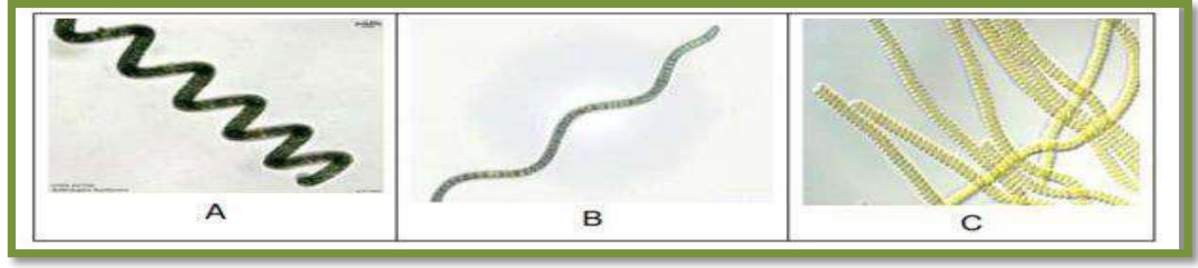

Figure 3: Various microscopic aspects of *Spirulina* **(Goulamabasse, 2018).**

(A) Spiral shape, (B) Wavy shape, and (C) Straight shape.

I.5 Habitat and Geographic

Spirulina preferentially grows in warm, alkaline waters that are rich in nitrogen and phosphorus nutrients. It is commonly found in brackish waters and salt lakes in tropical and semi-tropical regions. Its thermophilic nature and high light requirements limit its distribution to an intertropical zone located roughly between 35 degrees north and 35 degrees south of the equator. *Spirulina* can be discovered in natural alkaline lakes throughout Africa (including Tchad, Ethiopia, and Tunisia), Latin America (Mexico and Peru), and South Asia (such as India, Sri Lanka, and Thailand), reflecting its significant ecological versatility. While considered to be present everywhere, *Spirulina*'s levels are notably lower in North America and Europe **(Charpy *et al., 2008*)** (fig.4).

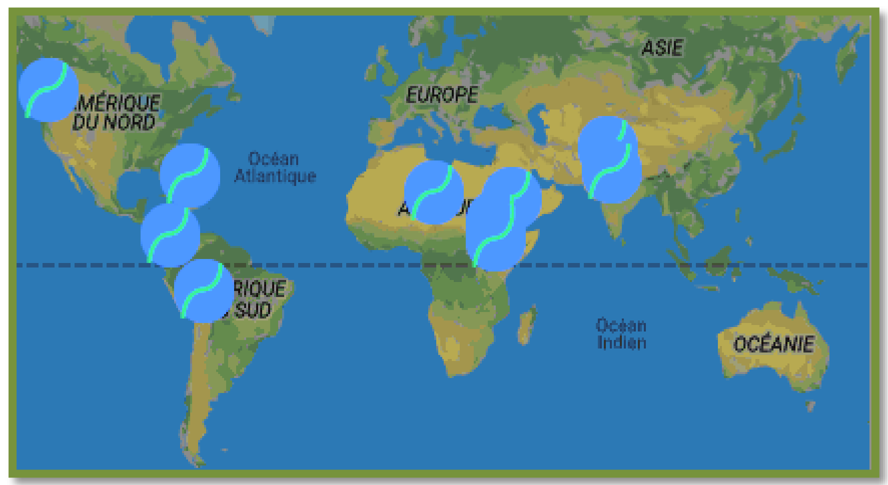

Figure 4: Geographic distribution of *Spirulina* **(Fox, 1999)**

I.6 Life cycle and Reproduction

Spirulina reproduces through a vegetative mode, an asexual multiplication that follows the principle of binary fission. It is thus a simple division by segmentation of filaments, which occurs in several stages.

- Once maturity is reached, the *Spirulina* filaments form necridia, cells with a concave appearance.
- This is followed by fragmentation of the trichome from the necridia, resulting in new filaments composed of 2 to 4 cells called hormogonia.

These hormogonia grow by binary division and take on the typical helical shape, with each cell giving rise to two cells through binary fission (Fig. 5) **(Manet, 2016).**

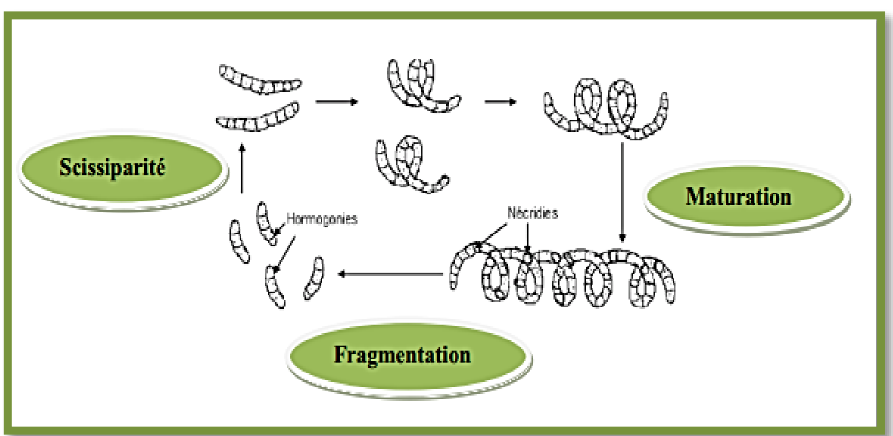

Figure 5: Biological reproduction cycle of *Spirulina* (**Charpy** ***et al., 2008*****).**

I.7 Cultivation and production of *Spirulina*

I.7.1 Cultivation of *Spirulina*

Spirulina platensis is a widely cultivated cyanobacterium in the world. The world production of this first can exceed 3000 tons of biomass per year **(Benahmed, 2012).**

I.7.1.1 Culture Parameters

One of the greatest advantages of cultivating *Spirulina* compared to other types of cultures is its growth under extreme conditions (salinity and alkalinity), thus making it possible to exclude the proliferation of most other microorganisms **(Benahmed, 2012).**

I.7.1.1.1 Temperature *Spirulina* is a mesophilic cyanobacterium that grows at temperatures varying between 25 and 40°C **(Fox, 1999),** with an optimum of 30°C **(Ogbonda** ***et al., 2007*****).** At temperatures below 20°C, the growth rate is slowed down or practically stopped **((Jourdan, 2006) ;(Ogbonda** ***et al., 2007*****)).** Beyond 40°C, the culture will wither away from excess heat and the *Spirulina* will eventually die **(Benahmed, 2012).**

I.7.1.1.2 Hydrogen potential (pH) *Spirulina* proliferates in alkaline waters whose pH varies between 8.5 and 11 with an optimum of 9.5 **((Ciferri, 1983).; (Fox, 1999)).** Alkalinity is usually provided by sodium bicarbonate (NaHCO3), but the latter can be replaced, in part; by caustic soda or sodium carbonate (Na2CO3) to raise the initial pH of the culture medium **(Fox, 1999).**

I.7.1.1.3 Salinity The growth of ***Spirulina*** Seems to be directly linked to the concentration of salts in the medium. It grows in saline waters at concentrations varying between 20 and 70 g/l **(Ciferri, 1983).**

I.7.1.1.4 Light *Spirulina* Is a photosynthetic organism, to grow it needs light with an intensity between 30 and 40 klux. However, at high light intensities, photolysis may occur **(Benahmed, 2012).**

I.7.1.1.5 Oxygen and agitation Like any other aerobic microorganism, *Spirulina* needs oxygen (O2) to breathe. However, this O_2 can be toxic when it is oversaturated during active photosynthesis **(Jourdan, 2006).** During the day, agitation is necessary to homogenize, promote the elimination of oxygen and ensure a good distribution of light **(Jourdan, 2006).**

I.7.2 Production of *Spirulina*

The production of *Spirulina* is done on several scales: artisanal, semi-industrial and industrial.(Tab.2)

Table 2: Various *Spirulina* productions and their characteristics **(Charpy *et al., 2008*).**

Features	Artisanal	Semi-industrial	Industrial
Size of basins	< 100 m²	200 - 1000 m²	1000 m² -5000 m²
Total exploited surface	< 3000 m²	3000 m² - 1 hectare	Several hectares
Annual production capacity	< 200 kg	10 – 50 tonnes	>50 tonnes

I.7.2.1 Artisanal production

Historically, this mode of cultivation was initiated by Ripley FOX to fight against malnutrition in developing countries. In recent years, this mode of production has continued to grow, supported by many NGOs. These are systems requiring low energy inputs. The means implemented can be rustic, appealing to good engineering sense. Nevertheless, some artisanal farms may present semi-industrial farm characteristics. Production quality is controlled throughout production. They are intended for humanitarian aid or partly for marketing **(Charpy *et al.,2008*).** (Fig.6).

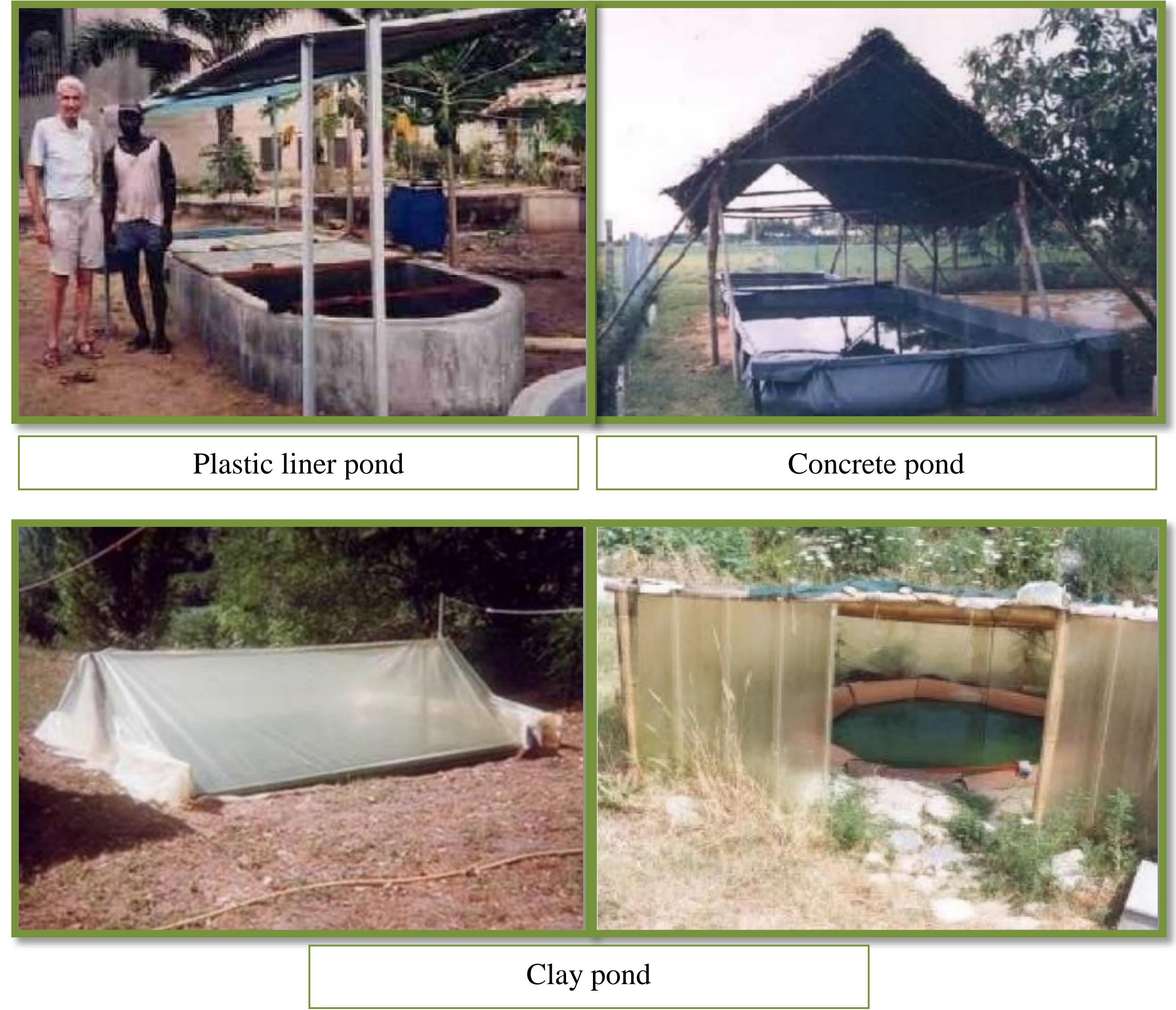

Figure 6: Various artisanal cultures of *Spirulina* **(Jourdan,2018).**

I.7.2.2 Semi-industrial production

In developing countries, semi-industrial farms use the same technologies as artisanal farms. They are intended for humanitarian and commercial purposes. Their goal is to be sustainable and autonomous through the sale of their product **(Charpy *et al., 2008*).**

I.7.2.3 Industrial production:

Represented for more than twenty years by large companies such as Earthrise or Cyanotech, they are distinguished from the previous ones by the importance of the means implemented, their production capacity and their clearly commercial objective. Quality is controlled automatically by computerized systems **(Charpy *et al., 2008*).**(Fig.7)

Figure 7: Improved basins in Hawaii **(Théodore,2017)**

I.8.Main applications of *Spirulina*

I.8.1 In human food

Spirulina is primarily known for its use as human food and is highly regarded for its exceptional nutritional profile, offering several benefits. Humanitarian workers and doctors use it in powder form to combat severe malnutrition in children, and it has been found to be more effective than drugs in treating deficiencies and diseases such as starvation, kwashiorkor, protein-energy malnutrition, iron deficiency anemia, and hypovitaminosis. For athletes, *Spirulina* facilitates effort and aids in recovery due to its high content of vitamins B9 and B12 as well as iron. Pregnant women can also benefit from *Spirulina* consumption, as it contains phycocyanin, which increases muscle oxygenation and limits uterine cramps, allowing for better preparation for childbirth and post-breastfeeding recovery. *Spirulina* is highly suitable for children and adolescents, as well as for babies of age to consume protein, due to its high assimilability and essential quality elements. It also adds to the quality of the skin. In the field of dietetics, *Spirulina* is used as a protein supplement beneficial for health, acting as an appetite suppressant, reducing appetite, and optimizing energy intake. Just three to five grams per day of *Spirulina* can help avoid deficiencies and eliminate toxins related to fast food consumption by teens **(Toudert and Bouzidi, 2020)**

I.8.2 In cosmetics

Spirulina, due to its high concentration of natural active ingredients such as amino acids, trace elements, antioxidants, minerals, vitamins, nucleic acids, proteins, and essential fatty acids, has been introduced by some cosmetic care laboratories into their marketed creams, shampoos, and serums. Its antioxidant properties make it beneficial in improving skin flexibility and elasticity,

delaying aging, and providing shine and resistance to nails and hair. It is considered an exceptional" beauty" food and used in various anti-aging treatments with a marine connotation, spa and thalassotherapy products such as face masks, body wraps, poultice, marine wrapping, body conditioner, and face mask for its restorative and fortifying effects on hair and nails **(Banks,2007).**

Table 3: *Spirulina* benchmark on skin care products **(Ragusa *et al, 2021*).**

Cosmetic Product	INCI *Spirulina*	Key Benefits/Claims	Company	Product's Photo
KELLY powder mask	*S.platensis* powder	Peel-off for dry skin	PuroBIO cosmetics (Bari, Italy)	
Mattifying, purifying clay mask	*S.platensis* powder	Effective against blemishes. It minimizes the appearance Effective against blemishes. It minimizes the appearance of pores, removes excess sebum and fights congestion, without drying the skin out	REN Skin care (UK)	
Powercell Skinmunity Emulsion	*S. platensis* extract	Stimulates revitalization, smoothes wrinkles, intensely hydrates the skin	HELENA RUBINSTEIN (Australia)	
Face cream, face serum	*S. platensis* extract	Nourishing, revitalizing, moisturizing, antioxidant,ditox	SUKIN SKINCARE (Australia)	

I.8.3 In medicine

The unique composition of *Spirulina* has led to numerous therapeutic applications, including strengthening the immune system to fight opportunistic diseases and treating certain skin conditions. It has also been shown to be effective in relieving rheumatic pain, osteoarthritis, osteoporosis, excess cholesterol, hypertension, and allergies, while also protecting the heart and promoting the regeneration of brain cells. *Spirulina* is now available as a dietary supplement for these therapeutic purposes **(Boudaoud, 2016).**

I.8.4 In animal feed

Spirulina is not only beneficial for humans but also for animals. It enhances their natural defenses and helps to maintain their immune system, which aids in fighting certain diseases and combating aging and fatigue. Dogs, cats, fish, and horses are some of the common animals for which *Spirulina* is used. In horses, it is frequently consumed during growth, competition, or convalescence phases. Poultry farmers often add *Spirulina* to chicken feed to enhance egg laying quality, a practice that is well-known among experienced farmers **(Casal, 2019).**

I.8.5 In agri-food

It is used as a natural colorant (phycocyanin is one of the few natural blue pigments) in chewing gum, sorbets, candies, dairy products, and carbonated beverages. It is also found in many products based on algae combined with salt, tagliatelle, etc. *Spirulina* bread has been available in Switzerland and Japan for a long time **(Boudaoud, 2016).**

CHAPTER II. BIOCHEMICAL COMPOSITION OF *SPIRULINA*

II.1 Composition of *Spirulina platensis*

Spirulina is a biomass extracted from cyanobacteria that both humans and animals can consume. Its use does not stop at the nutritional supplement, but it can be a complete food. It is effectively used as a food supplement in aquaculture, fish ponds and poultry **(Robin, 2017).**

The composition of *Spirulina* varies according to the cultivation conditions, the harvesting period, the geographical origin, the harvesting, drying, grinding, and packaging process, as well as the rate of sunlight exposure **(Manet, 2016).**

Spirulina contains between 60% and 70% protein, 15% carbohydrates, 6% lipids, 7% minerals and 3% to 6% water in its composition (Fig. 8) **(Niangoren, 2017).**

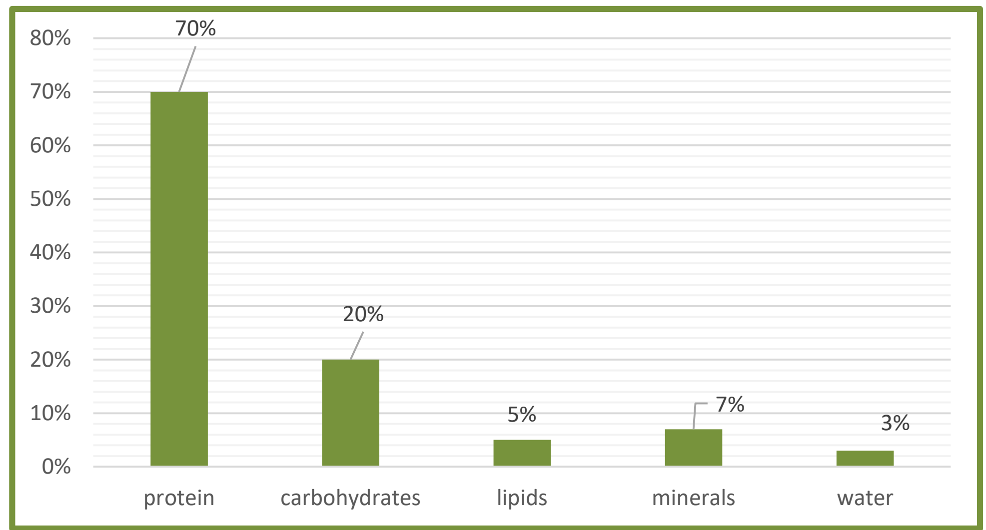

Figure 8: Average chemical composition of *Spirulina* **(Goulambasse, 2018).**

II.1.1 Protein

From a qualitative perspective, *Spirulina* protein is complete, therefore of high quality, as all essential amino acids are present **(Jourdan, 2006; Gutierrez *et al., 2015*).** They are almost 100% bioavailable, meaning that the body can use almost all of it. This is partly what makes these microalgae nutritionally rich **(Barth *and Léo, 2019*).**

The protein content of *Spirulina* is high, with variations of 10 to 15% depending on the harvesting time. The higher the brightness, the higher the percentage of protein. It represents 10 to 11% of the wet mass, or 60 to 70% of its dry matter. This percentage is much higher than that of fish (25%), soy (35%), milk powder (35%) and cereals (14%) **(Goulambasse, 2018).**

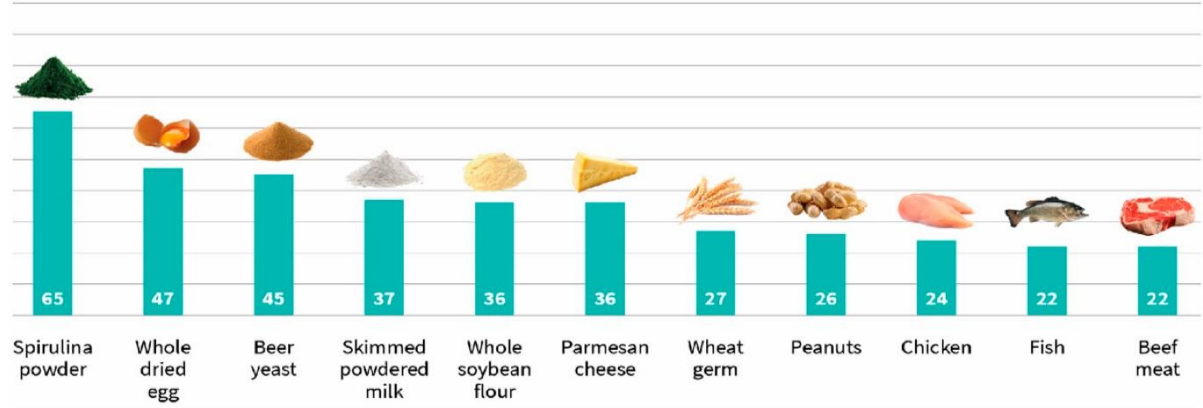

Figure 9: Positioning of *Spirulina* in relation to other foods in terms of protein levels (%) **(Vrenna *et al., 2021*).**

II.1.2 Lipids

II.1.2.1 Total lipids

The total lipids represent less than 10% of the dry weight. These total lipids can be separated into a saponifiable fraction (83%) and an unsaponifiable fraction (17%) containing mainly paraffins, pigments, terpenic alcohols, and sterols **(Clement., 1975).**

II.1.2.2 Fatty acids

Essential fatty acids are polyunsaturated fatty acids classified into two groups (omega-3 and omega-6), based on the position of the nearest unsaturation to the terminal methyl group. Omega-3 fatty acids play a preventative role in cardiovascular risks, while omega-6 fatty acids have a hypocholesterolemic role **(Cruchot., 2008).**

II.1.3 Carbohydrates

Carbohydrates represent between 15 and 25% of dry matter. Simple carbohydrates are present in very low quantities (glucose, fructose, and sucrose), while assimilable carbohydrates are primarily composed of polymers such as amino glucosanes (1.9% of dry weight), amino rhamnosanes (9.7%), and glycogen (0.5%) **(Quillet., 1975).**

From a nutritional standpoint, the only interesting carbohydrate substance in *Arthrospira* in terms of quantity is meso-inositol phosphate, which is an excellent source of organic phosphorus and inositol (350-850 mg/kg of dry matter). Polysaccharides have multiple therapeutic benefits, particularly in stimulating DNA repair mechanisms, its radioprotective effect, and neutralizing free radicals **(Boudour., 2008).**

II.1.4 Vitamins

II.1.4.1 Beta-carotene (provitamin A)

Beta-carotene accounts for 80% of the carotenoids present in *Arthrospira*, with the rest being mainly composed of physoxanthine and cryptoxanthine **(Palla *et al., 1969*).** There are between 700 and 1700 mg of beta-carotene and approximately 100 mg of cryptoxanthine per kilogram of dried *Spirulina*.

II.1.4.2 Tocopherols (vitamin E)

From 50 to 190 mg per kilogram, **(Challem *et al., 1981*),** a comparable content to that of wheat germ. The daily requirements for vitamin E would be 15 I.U. **(Guyton, 1986)** equivalent to 12 mg of free tocopherols.

II.1.4.3 Group B vitamins

Although less rich in B-group vitamins (except for B12) than yeast, *Arthrospira* is still a good source of these cofactors (Table 4).

Table 4 : Vitamin content **(Falquet, 1996).**

Vitamin	Content (mg/kg)	Need/day (adult)
Thiamine (B1)	34-50	1.5mg
Riboflavin (B2)	30-46	1.8mg
Pyridoxine (B6)	5-8	2.0mg
Cyanocobalamin (B12)	1.5 - 2.0	0.003 mg
Niacin	130	20mg
Folate	0.5	0.4mg
Panthotenate	4.6 - 25	6 - 10 mg
Biotin	0.05	0.1 - 0.3mg
Ascorbic acid (C)	Traces	5 - 30 mg

II.1.4.4 Vitamin B12

We must emphasize the exceptional content of vitamin B12 (cobalamin), which is by far the most difficult to obtain in a meatless diet because no common vegetable contains it. *Arthrospira* is four times richer than raw liver, long given as the best source. It should be noted, however, that there is a controversy about the real bioavailability of the B12 complex of *Arthrospira* **((Hayashi *et al., 1996*); (Rule *et al., 1994*)).**

II.1.5 Minerals

During cultivation, *Spirulina* absorbs several minerals from the medium. Thus, its content in minerals varies depending on the growth medium and the minerals present in the water **(Henrikson, 1997).**

The minerals of particular interest in *Arthrospira* are magnesium, calcium, phosphorus, potassium, iron and zinc. The first three are present in the *Spirulina* at levels comparable to those found in milk **(Falquet,1996).**

It is worth noting the very high iron content, as well as the presence of selenium and fluoride, which have certain positive effects (fighting free radicals, preventing dental caries) **(Falquet, 1996).**

Table 5 : Mineral content **(Falquet, 1996)**

Mineral	Content (mg/kg)
Calcium	1300 – 1400
Iron	580 – 1800
Phosphorus	6700 – 9000
Zinc	21 – 40
Magnesium	2000 – 2900
Copper	8 – 10
Sodium	4500
Potassium	6400 -15400
Manganese	25 – 37

II.1.6 Nucleic acids

Nucleic acids represent 4 to 6% of the dry matter. The proportion of DNA would be a quarter to one-third compared to RNA. The nucleic acid content of *Arthrospira* is much lower than that of unicellular generality **(Ciferri, 1983).**

Table 6: Nucleic acid content **(Borwitzka, 1988).**

Food	Nucleic acids (% dry matter)
Beef	1.5
Beef liver	2.2
Spirulina	4-6

II.1.7 Pigments

Spirulina, also known as blue-green algae, owes its name to the presence of phycocyanin, which is the most valuable pigment found in *Spirulina*. However, we can also see green and sometimes orange color in *Spirulina*, which is likely due to the presence of other pigments such as chlorophyll and carotenoids.

Table 7: Pigments in *Spirulina* powder **(Ali and Saleh, 2012)**

Pigment	Content in mg/10g
Phycocyanine (blue)	1500-2000
Phycoerythrine (red)	2900-10000
Chlorophylle a (green)	61-75
Carotenoids (orange)	37

II.1.7.1 Chlorophyll

Spirulina contains approximately 1% of chlorophyll, which is one of the highest concentrations found in nature. Its structure is closely related to mammalian hemoglobin, which has earned it the nickname of" green blood". Despite not being the most abundant pigment in *Spirulina*,chlorophyll is responsible for its green color due to its strong coloring power. Chlorophyll has been the subject of numerous studies and has been shown to have several beneficial qualities, including restoring acid-base balance, improving cardiac function, regulating intestinal transit, increasing red blood cell count, and promoting internal and external healing. **(Casal, 2019).**

II.1.7.2 Phycocyanin

Phycocyanin, which gives *Spirulina* its characteristic bluish color, is considered the most remarkable pigment present in *Spirulina*. **(Sguera, 2008)** It is known to be the most potent antioxidant and anti-radical substance available, providing a boost to the body's natural defenses. Phycocyanin has been found to stimulate the production of red blood cells, promote muscle activity, inhibit the growth of cancer cells, and eliminate harmful chemicals from the body **(Gabr *et al., 2020*).**

II.1.7.3 Beta-carotene

Beta-carotene is an orange pigment and a precursor of vitamin A that is abundant in *Spirulina*. It plays a vital role in cell renewal and supports immune defenses, as stated by **(Charpy *et al., 2008*).** This nutrient has numerous antioxidant properties that combat cell aging, reduce the risk of cancer, promote wound healing, and protect the skin from external aggressors, according to **(Asghari *et al., 2016*).**

II.1.8 Enzymes

Numerous enzymes also make up *Spirulina*, biological "facilitators," including the exceptional SOD (superoxide dismutase), which is a major weapon against cellular oxidation or aging **(Ahounou, 2018).**

CHAPTER III.
BIOLOGICAL ACTIVITIES OF *SPIRULINA*

III.1 Antioxidant activity

III.1.1 Oxidative stress

In recent years, antioxidants have become the subject of several studies in medical biology. What has attracted researchers' attention is the discovery of a phenomenon concerning all degenerative processes: under the influence of the sun, stress, tobacco, pollution, or an unbalanced diet, our cells can malfunction, and sometimes DNA can be damaged; leading to mutated cells that can cause cancer. This phenomenon is now known as oxidative stress, which is facilitated by the presence of free radicals.

III.1.1.1 Free radicals

Free radicals are chemical substances with a single free electron carried by an oxygen atom. The presence of a unique electron gives them a high level of instability. They are known as "reactive oxygen species" (ROS) or "reactive oxygen derivatives" (ROD), and sometimes "oxygen-derived radical species". In some cases, the free electron is carried by a nitrogen atom, and they are known as "reactive nitrogen species" (RNS). ROS and RNS can be generated in cells during metabolism by various endogenous systems. They play an essential role in fighting viruses and bacteria, but with a certain amount, they become very reactive and attack neighboring molecules **(Goudable and Favier., 1997).**

Oxidative stress is a state of imbalance between the production of reactive oxygen species and reactive nitrogen species on one hand, and the body's ability to prevent oxidative damage on the other hand. There are numerous diseases associated with oxidative stress, including Alzheimer's, Parkinson's disease, atherosclerosis, cardiac hypertrophy, diabetes, heart failure, hypertension, some cancers, and organ aging **(Wu *et al.,2016*).**

III.1.1.2 Antioxidants and defense systems

Through the use of antioxidants, the body protects itself against oxidative stress. Antioxidants act as scavengers of free radicals, and there are two types:

III.1.1.2.1 Endogenous antioxidants

These are antioxidant enzymes or proteins (Superoxide dismutase, Catalase, and Glutathione peroxidase) produced by our body with the help of certain minerals. They are constantly present in the body, but their quantity decreases with age **(Meyer and Deiana, 1988).**

III.1.1.2.2 Exogenous antioxidants

Exogenous antioxidants are present in the diet such as vitamins A, C, E and polyphenols, as well as selenium, zinc and manganese **(Bougandoura, 2010).**

III.1.2 Antioxidant potential of *S. platensis*

Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported as dangerous to human health. Therefore, the search for effective and non-toxic natural compounds with antioxidant activity is essential.

Spirulina is an exceptional source of antioxidants; one ton of fruits contains the amount of antioxidants found in one kilogram of *Spirulina* **(Manet,2016).** It is rich in phycocyanin, β-carotene, vitamin A, C, E, SOD enzyme, gamma-linolenic acid, selenium, methionine, etc. Moreover, the activities of most endogenous antioxidants were increased after *Spirulina* administration **(Moor *et al* 2020),** several scientific studies have demonstrated the antioxidant capacity of *Spirulina*.

III.2 Other activities

III.2.1 Antibacterial activity

Preliminary in vitro studies have shown that *Spirulina* extracts exhibit effective antimicrobial potential against some pathogenic bacteria such as *E. coli* and *S aureus*.

This suggests that cyanobacteria possess a defense mechanism to fight against pathogenic bacteria, which could potentially lead to the development of plant-based antibiotics with fewer side effects than synthetic drugs. The antibacterial activity of *Spirulina* can be attributed to the presence of its various bioactive compounds, such as alkaloids, flavonoids, steroids, saponins, and tannins **(Fithriani *et al.,* 2015; Setyaningsih *et al.,* 2020).** Each of these compounds has recognized antimicrobial properties, and their combined presence contributes to the overall effectiveness of *Spirulina* against a variety of pathogens.

III.2.2 Anti-inflammatory activity

The immune system is the primary defense mechanism against physical infections, and cytokines play a central role in initiating inflammation. Pro-inflammatory cytokines, such as TNF α, IL-1β, and IL-6, are produced when the immune system is activated. The benefits of *Spirulina* in

enhancing immunity and improving resistance to inflammatory responses have been extensively documented. **(Charpy *et al., 2008*)**

COX-2 is the main form of cyclooxygenase involved in inflammation, responsible for producing prostaglandins at the site of inflammation. In a full human blood test, Vikocyanin, found in *Spirulina*, was found to significantly inhibit COX-2 with an IC50 value of 80 nm. Phycocyanin's ability to counteract inflammation is partly due to its selective inhibitory effect on COX-2, in addition to its ability to effectively eliminate free radicals and inhibit lipid peroxidation **(Charpy *et al., 2008*).**

Furthermore, *Spirulina* is rich in proteins and fatty acids, particularly omega-3 and 6, which the body cannot produce on its own. This makes it biologically significant since these fatty acids serve as precursors to prostaglandins, molecules with anti-inflammatory and immune activity within the body **(Charpy *et al., 2008*).**

III.2.3 Antiviral activity

The richness of *Spirulina* in β-carotene, vitamin B12, as well as other B group vitamins, which have long been established as interesting substances in the fight against viral infections, does not fully explain the antiviral power of *Spirulina*. It appears that the membranous polysaccharides of this algae are also involved in this process **(Andreani, 2012).** The efficacy of the polysaccharides against the replication of several enveloped viruses, such as Herpes Simplex Virus (HSV), influenza virus, measles virus, human cytomegalovirus (CMV), and HIV-l, has been demonstrated in vitro **(Yougbare, 2007).**

The mechanism appears to rely on the fact that the virus, unable to attach to the host cell membrane, cannot penetrate it, and as a consequence, cannot replicate **(Andreani, 2012).**

III.2.4 Anti-cancer activity

The development of certains types of cancer is often linked to damage to the DNA of cells, resulting in uncontrolled growth. While the body has enzymatic processes in place to identify and correct this damage, exposure to toxins can impair these processes and lead to the development of cancer. In addition to the development of therapeutic anti-tumor drugs, research

into cancer prevention has become a major focus, with a growing interest in chemical prevention using synthetic or natural substances that can inhibit carcinogenicity. One such substance is beta-carotene, an antioxidant found in *Spirulina*, which has been shown in various studies to potentially

reverse the cancer process and prevent the spread of cancer cells. In fact, a study conducted on individuals with precancerous oral lesions found that daily supplementation with 1 gram of *Spirulina* for a year resulted in an improvement in their condition and prevented the progression of the disease. Phycocyanin, another component of *Spirulina*, has also been found to contribute to this anti-cancer activity by attacking free radicals that are known to promote cancer growth **(Vidalo, 2015).**

III.2.5 Anti-hypercholesterolemic activity

The omega-3 and omega-6 fatty acids in *Spirulina* may prevent the accumulation of cholesterol in the body. This could partially explain the decrease in cholesterol and triglyceride levels observed in the experiments of Ramamoorthy & Premakumari in 1996 on 30 adult subjects with hypercholesterolemia. Additionally, *Spirulina* contains vitamin B3, also known as niacin, which is a hypocholesterolemic vitamin **(Ramamoorthy and Premakumari,1996).**

III.2.6 The therapeutic aspect

It is important to note that while *Spirulina* may have potential health benefits, more research is needed to fully understand its effects on various diseases and conditions. It is also important to consult with a healthcare professional before using *Spirulina* as a treatment or supplement, especially if you have any underlying health conditions or are taking medication. It is always best to follow the guidance of healthcare professionals and public health organizations when it comes to preventing and treating illnesses **(Elaya Perumal and Sundararaj, 2020).**

THE PRACTICAL PART

CHAPTER Ⅰ. MATERIALS AND METHODS

This work was carried out in the pedagogical laboratory of Microbiology of the Faculty of Natural and Life Sciences, 20th August 1955 University -SKIKDA-.

I.Materials

I.1. Biological Material

The biological material used in this study is a sample of dry biomass from the *Arthrospira platensis* strain cultivated on a biological farm in Biskra region (Biofarm Al-Kiram). This material was chosen primarily for its richness in bioactive compounds. The sample of *Spirulina* we acquired is presented in the form of flakes, packaged in a 100 g bag. We grind the sample using a mortar to obtain a fine *Spirulina* powder (Fig.10).

Figure 10: *Spirulina* powder

I.2. Non-biological material and equipment

This refers to any material, other than biological, that includes glassware, apparatus and chemical and organic reagents used in the experimental study.

I.2.1. Microscopic observation of *Spirulina*

Table 8: Tools, apparatus, solutions and reagents used in the characterization of *Spirulina* strain.

Tools	Solutions / Reagents	Apparatus
Biological material Beakers Slide and coverslip Pasteur pipette	Distilled water Crystal Violet Iodine Ethanol Fuchsine Immersion oil	Optical microscopic Analytical balance

I.2.2 Preparation of extracts

Table 9: Tools, apparatus and solutions used in preparation of extracts.

Tools	Solutions / Reagents	Apparatus
Biological material Beakers Spatula Graduated cylinder Wattman Filter Paper Aluminum foil Petri Dishes	Ethanol Distilled water	Analytical balance Magnetic stirrer Rotary evaporator Oven

I.2.3 Determination of pigments contents (Chlorophyll a, b and Carotenoids)

Table 10: Tools, apparatus and solutions used to determination of pigments contents.

Tools	Solutions / Reagents	Apparatus
Biological material Graduated cylinder Beaker	Acetone 90%	Analytical balance Magnetic stirrer Refrigerator Centrifuge Spectrophotometer

I.2.4 Extraction of phycocyanin

Table 11: Tools, apparatus and solutions used to extraction of phycocyanin.

Tools	Solutions / Reagents	Apparatus
Biological material Graduated cylinder Beakers Wattman Filter Paper Aluminum foil	Phosphate buffer solution Distilled water Glycerol	Refrigerator Centrifuge Spectrophotometer

I.2.5 Evaluation of antioxidant activity

Table 12: Tools, apparatus and solutions used in the evaluation of antioxidant activity.

Tools	Solutions / Reagents	Apparatus
Sterile Test Tubes Beakers Micropipette Graduated cylinder Aluminum foil Tubes holder	Extracts DPPH Solution (2,2-diphenyl-1-picrylhydrazyl) Ethanol Ascorbic acid Distilled water	Analytical balance Magnetic stirrer Spectrophotometer

I.2.6 Determination of the antibacterial activity

Table 13: Tools, apparatus and solutions used in the determination of the antibacterial activity.

Tools	Solutions / Reagents	Apparatus
Preserved bacterial strains Sterile Test Tubes Platinum loop Sterile Petri Dishes Tubes holder Swabs Sterile discs Sterile forceps Micropipette	Extracts Nutrient broth Nutrient agar Chapman agar Hektoen agar Muller Hinton agar Physiological saline DMSO	Bunsen burner Oven Autoclave

II.Methods

II.1. Microscopic observation of *Spirulina*

II.1.1. Wet mount examination

- **Procedure**

1. The preparation of suspension was carried out by taking a small amount of *Spirulina* powder using a sterile spatula, mixing the powder with a small amount of sterile distilled water in a small beaker, and gently shaking until a homogeneous suspension was obtained.

2. Using a Pasteur pipette, a drop of the *Spirulina* suspension was placed on a clean slide.

3. A cover slip was gently applied to the drop of suspension to prevent the formation of air bubbles.

4. The slide was observed under the microscope (10X and 40X magnification).

II.1.2. Gram staining

- **Procedure:**

1. Preparation of the Smear:

A small amount of the *Spirulina* suspension was taken and spread on a clean slide.

The smear was air-dried and then heat-fixed.

2. Staining:

The smear was stained with Crystal Violet for 1 minute, then rinsed.

Iodine solution was applied for 1 minute, then rinsed.

3. Decolorization:

The smear was washed with alcohol briefly, then rinsed immediately.

4. Counterstaining:

The smear was stained with Fuchsin for 1 minute, then rinsed.

5. Drying and Observation:

The slide was blotted dry.

The slide was observed under a microscope using oil immersion (100x magnification) **(Murray *et al.,2015*).**

II.2. Preparation of extracts

Maceration extraction is one of the most commonly used extraction methods for obtaining secondary metabolites from medicinal plants.

Maceration involves allowing a solid to soak in a liquid to extract the soluble constituents in that liquid.

The extraction was carried out by mixing 10g of *Spirulina* powder with two types of solution:

- 100 mL of ethanol
- Mixture of 100 mL of ethanol/water (70/30: v/v)

The mixture was stirred at room temperature for 60 minutes, left to rest in the dark at room temperature for 72 hours to deplete the plant material and collect the maximum compounds, then filtered using Whattman paper.

The obtained extract was concentrated under vacuum at 70˚C by a rotary evaporator until the evaporation of ethanol. The extracts were transferred to a hot air oven, where they were dried at 40 °C and stored in a dark bottle at 4 °C. A portion of the extract was used for the evaluation of antioxidant activity, while the rest was used for the bacterial sensitivity test. **(Ali and Doumandji, 2017).**

II.2.1. Calculation of dry extract yields

The extraction yield corresponds to the percentage of the active ingredient dissolved in the organic solvent used for the extraction. It is determined based on the weight of the dry extract in relation to the weight of the dry plant material used for the extraction **(Abe *et al.*, 2010).**

The yield is expressed as a mass percentage relative to the amount of dry matter using the formula:

$$\mathbf{R\ (\%) = [M_1 / M_0] \times 100.}$$

R %: Extract yield expressed in g/100g of dry matter.

M_1: Quantity of extract recovered expressed in g.

M_0: Quantity of plant powder used for the extraction expressed in g **(Abe *et al.,* 2010).**

The experimental device adopted for the realization of this part of the work is represented in figure 11:

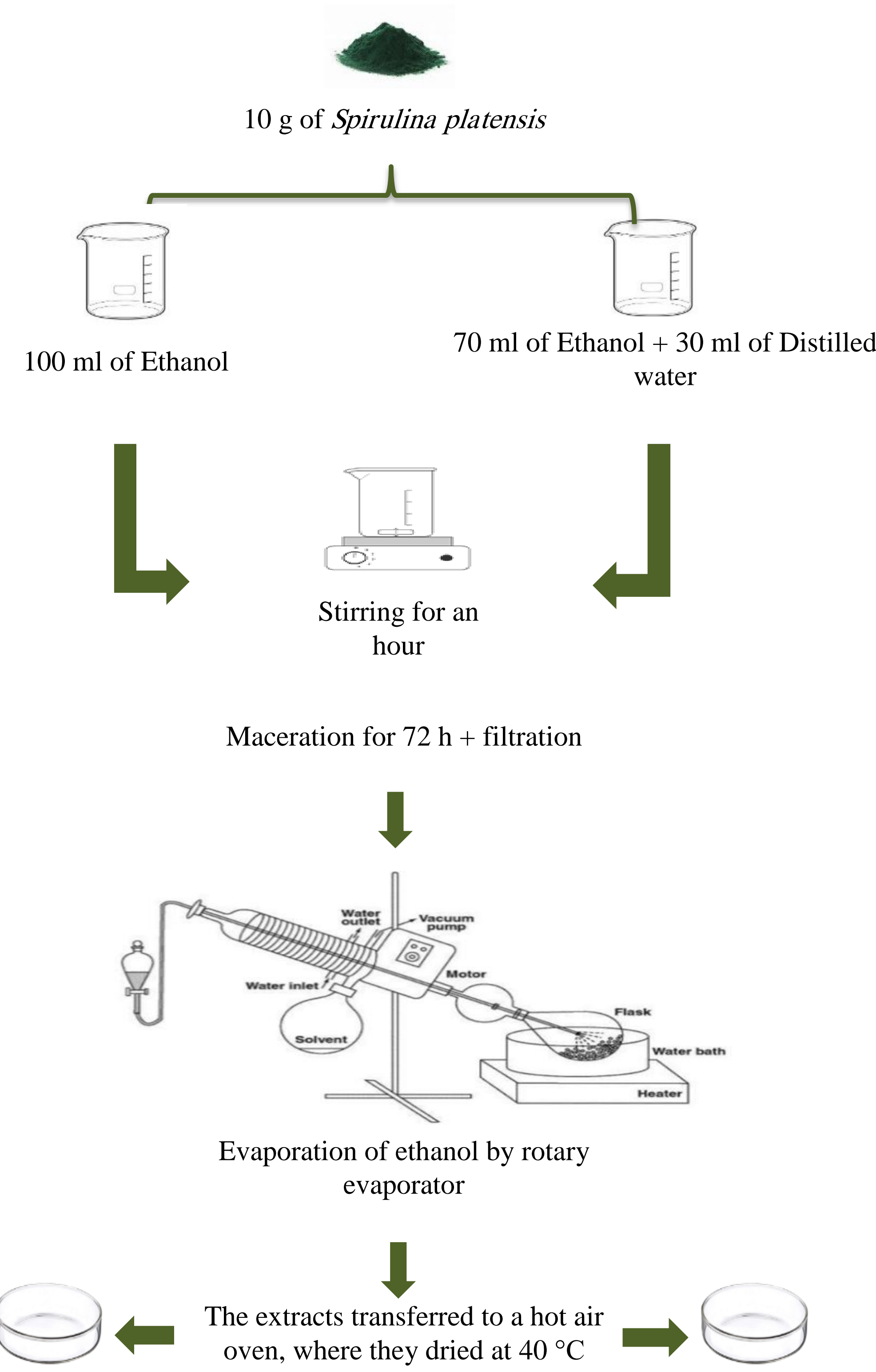

Figure 11: Ethanol and hydro-ethanol extraction protocol from *Spirulina* powder by maceration.

II.2.3. Determination of pigments contents (Chlorophyll a, b and Carotenoids)

A. Principle

The isolation of new chlorophyll degradation products remains the key step in understanding the mechanisms of the chlorophyll degradation phenomenon. This is why it will be interesting to try to detect, isolate and characterize one (or more) catabolize(s) present in the *Spirulina* samples.

B. Operating mode :

Chlorophyll a, b and carotenoids were determined according to the modified method described by **(El-Sheekh *et al., 2009*)** The procedure involved the following steps:

- In this process, one gram sample of *Spirulina* is suspended in 50 ml of 90 acetone.
- Stirred vigorously with a magnetic stirrer (Stuart stir SB161, UK).
- The solutions were then placed in the dark at 4°C and centrifuged at 3900(x g) for 15 minutes.

C. Results expression

The supernatants obtained were used to determine the concentration of chlorophyll a (chla), chlorophyll b (chlb) and total carotenoids (Car) by measuring the absorbance at wavelengths 663.2,646.8 and 470 nm. The optical density is read with a UV/Visible spectrophotometer (Jenway Genova plus, Staffordshire, United Kingdom). The content (mg/g) of each pigment was quantity using equations (01-03) **(Soni *et al., 2018*):**

- **Equation 01 :**

$$Chla = 12.25 \cdot A663.2 - 2.79 \cdot A646.8$$

- **Equation 02 :**

$$Chlb = 21.5 \cdot A646.8 - 5.1 \cdot A663.2$$

- **Equation 03 :**

$$Car = (1000 \cdot A470 - 1.82 Chla - 85.02 Chlb) / 198$$

Where: A663.2, A646.8 and A470 are the respective absorbances of the sample at wavelength 663.2,646.8 and 470 nm. **(Soni *et al., 2018*)**

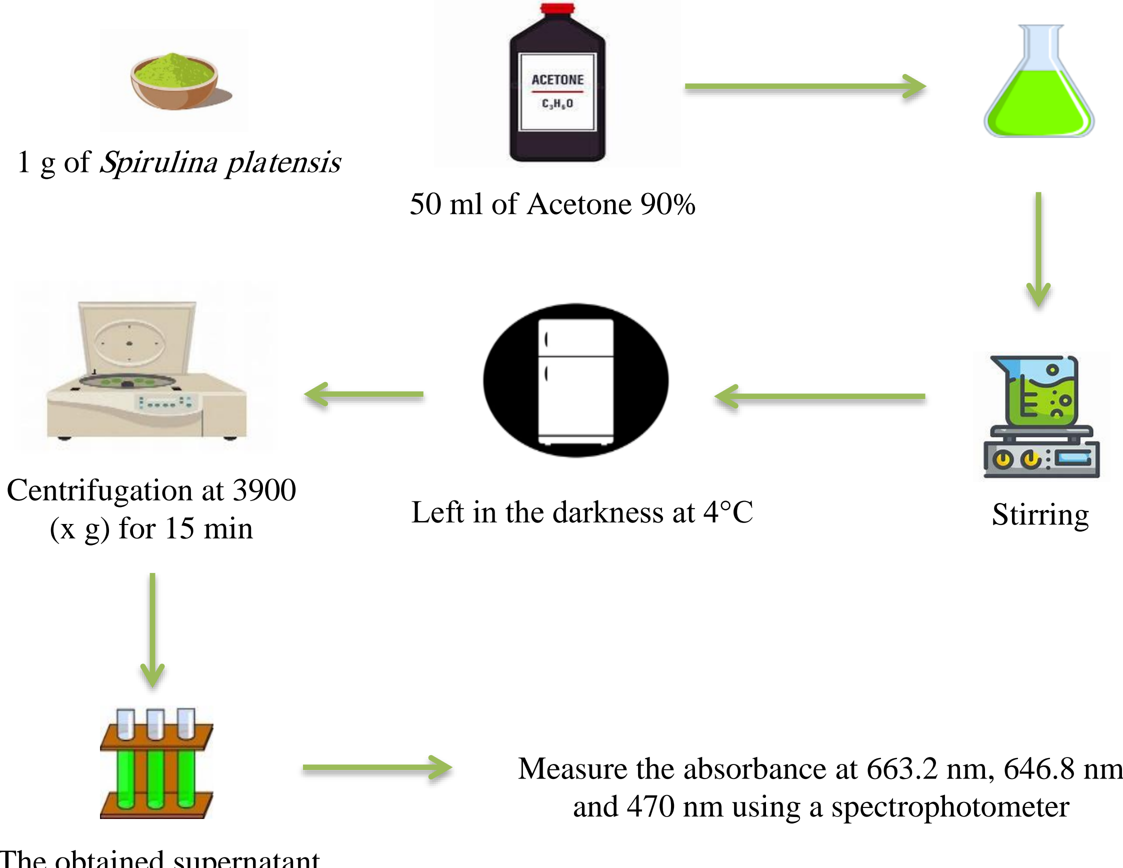

Figure 12: Protocol for determination of pigments contents (Chlorophyll a, b and Carotenoids)

II.2.4. Extraction of phycocyanin

II.2.4.1 Solvent Extraction

a) Principle

Solvent extraction can be used when a chemical species needs to be extracted from a solution in which it is dissolved. To extract a dissolved chemical species from a solution, another solvent is used (hence the term liquid-liquid extraction) that will also dissolve this species.

During the contact between the solution and the extraction solvent, the chemical species transfers from one to the other. Consequently, at the end of the extraction, we will obtain a new solution, following the protocol below:

b) Procedure

We add 4 g of *Spirulina* to 120 ml of phosphate buffer. Initially, we incubate it in the dark at 4°C for 12 hours (to allow cell lysis due to hypotonicity). Then, we centrifuge the mixture for the first time and collect the blue supernatant. Next, we add 120 ml of phosphate buffer to the precipitate and incubate for another 12 hours in the dark.

After the second centrifugation, the two supernatants are combined to measure the optical density at 615 nm, 652 nm, 620 nm, and 280 nm.

c) Expression of results

The calculation of the percentage of phycocyanin and its purity is based on the following equations (**Chen *et al.*, *2006***):

PC (mg/ml) = (A615-0.474xA652)/0.34

Purity = OD620/OD280

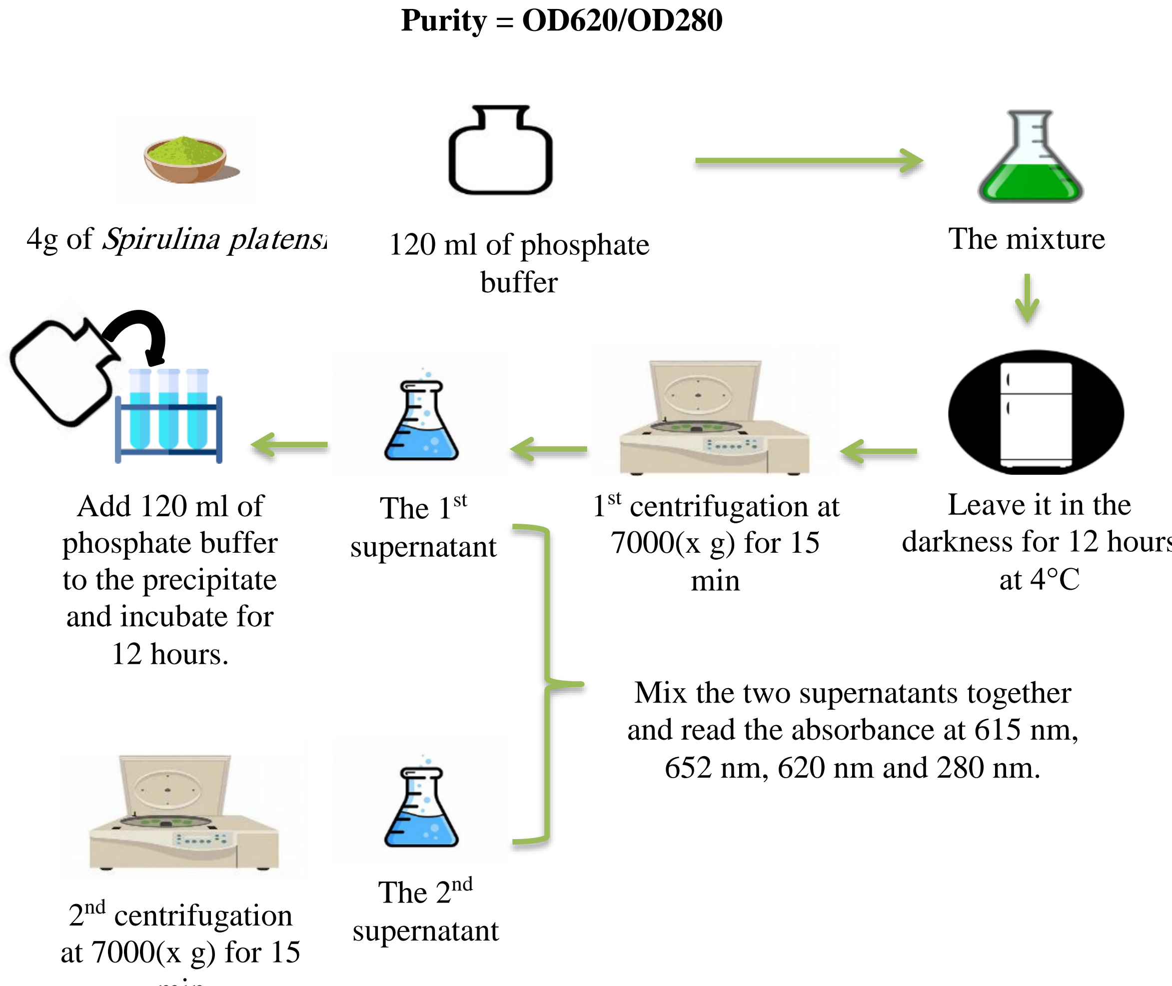

Figure 13: Protocol for extraction of phycocyanin by solvent

II.2.4.2 Extraction by maceration in glycerol

We followed a similar protocol to that of Lafri and his colleagues, with only the water/glycerol ratio being modified.

In this study, 50 g of dried *Spirulina* was mixed with a 50/50 water/glycerol ratio. The next step involved macerating the mixture for 15 days in the dark and at room temperature. Afterwards, the mixture was filtered using a 25 µm filter paper, and the percentage of phycocyanin was calculated using the following formula **(Lafri,2017):**

$$\mathbf{PC\ (mg/ml) = (A620 - 0.474 \times A650)/5.34}$$

After maceration and filtration, the obtained filtrate was centrifuged at 4000 rpm for 5 minutes. After being diluted 100 times, the absorption was then measured using a spectrophotometer.

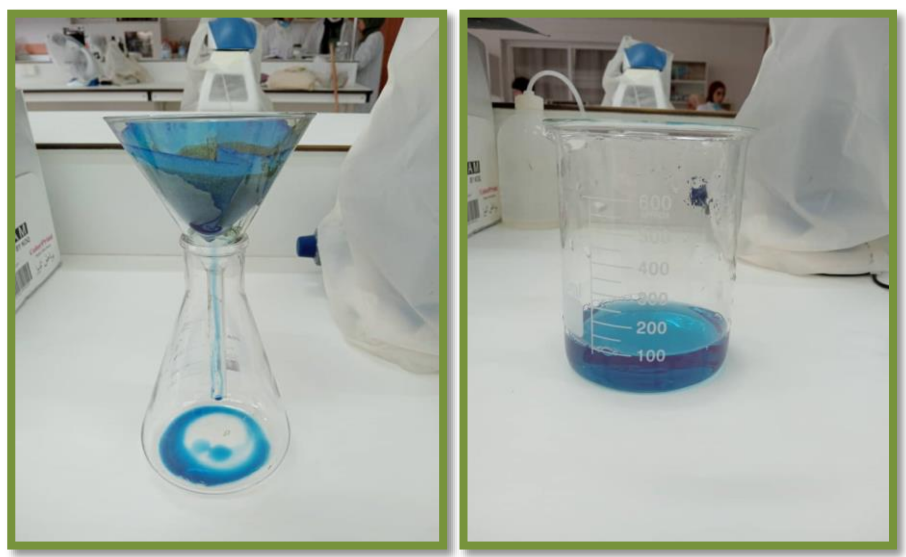

Figure 14: extraction process of phycocyanin by maceration in glycerol

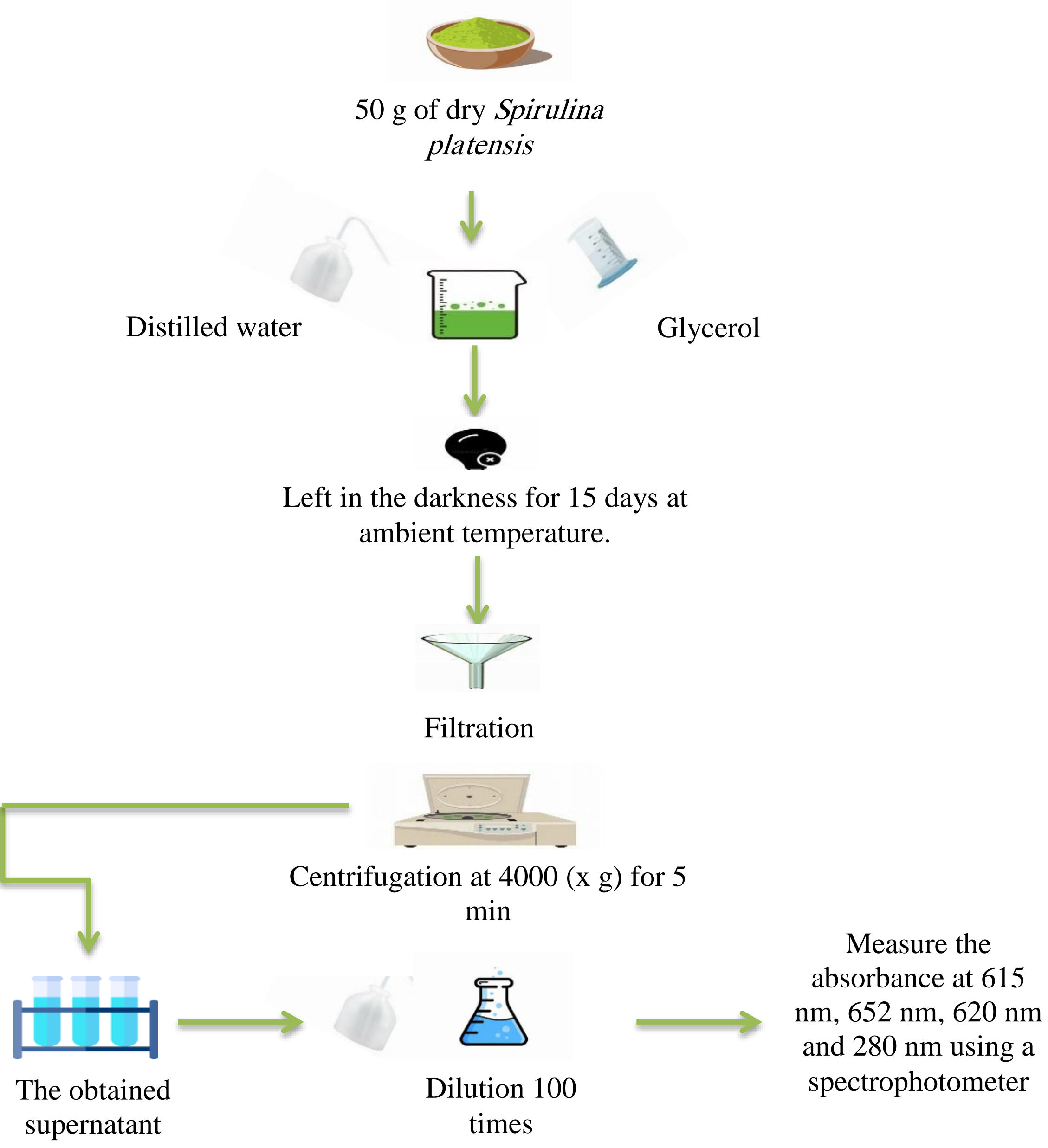

Figure 15: Protocol for extraction of phycocyanin by maceration in glycerol

III Study of biological activities

III.1. Evaluation of antioxidant activity

To estimate the antioxidant activity of the test extracts, we have the measure of the sample's ability to move radicals using the DPPH° (2.2-diphenyl-1picrylhydrazyl) root.(fig.16)

DPPH free radical inhibition test

- **DPPH root working principle**

The DPPH radical, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a solid substance with a black-violet color. However, when it is stabilized, it exhibits a yellowish orange color. The stability of the DPPH radical is attributed to the presence of aromatic rings in its structure, which allow for multiple resonant forms. This resonance delocalizes the electrons, preventing them from being confined to a single location. As a result, the DPPH radical remains stable for several days, exhibiting its characteristic color **(Mbaebie *et al., 2012*).**

- **DPPH free radical inhibition test**

The DPPH assay relies on the ability of extracts to donate hydrogen atoms to the DPPH free radical, resulting in its reduction. The reduction of the DPPH radical can be monitored using a spectrophotometer by measuring the decrease in absorption at a specific wavelength. This decrease in absorption indicates the ability of the extracts to inhibit or scavenge the DPPH free radical. By quantifying the reduction in absorption, we can assess the antioxidant capacity of the extracts and determine their effectiveness in inhibiting the DPPH free radical. **(Ardestani and Yazdanparast, 2007)**

a.Preparation of DPPH solution

Prepare the concentrated DPPH solution (0,4Mol/ml) by dissolving 4mg of DPPH in 100ml of ethanol **(Belguidoum *et al., 2015*).**

b.Preparation of concentrations

To prepare the mother solutions, 100 mg of each extract (ethanolic and hydro-ethanolic) was dissolved in 100 ml of ethanol and 100 ml of a mixture of distilled water and ethanol (30/70) to obtain a final concentration of 1 mg/ml. From this mother solution, further diluted concentrations were prepared by adding additional ethanol to the ethanolic extract and adding mixture of distilled water and ethanol (30/70) to the hydro-ethanolic extract, following the specified ratios. **(Belguidoum *et al., 2015*)**

c.Working Method

The antioxidant activity of the ethanolic and the hydro-ethanolic extracts is determined by assessing their ability to scavenge the DPPH free radicals. To measure this activity, different concentrations of extracts are prepared ranging from 0.2 to 1 mg/ml. The procedure involves mixing each extract at different concentrations with DPPH solution.

d.Calculation of the inhibition ratio for DPPH free root

The DPPH free root inhibition ratio for various concentrations of plant extracts and ascorbic acid is calculated according to the following formula:

$$I\% = (A_0 - A_i) / A_0 * 100$$

(I%): inhibition ratio.

A_0: Represents the absorption of the witness.

A_i: Represents sample absorption.

Using Microsoft Excel, we can create a chart depicting the percentage inhibition curve. By analyzing this curve, we can determine the concentration of the extract required to achieve 50% inhibition of the DPPH radicals. This concentration is referred to as the IC50 value, which represents the concentration necessary to inhibit 50% of the free radicals **(Belguidoum *et al., 2015*).**

To calculate the IC50 value, we utilize the equation derived from the inhibition ratio change curve (I%) in relation to the concentration of the extracts. The IC50 value is the concentration at which the inhibition ratio is 50%. By identifying the corresponding concentration on the x-axis of the chart where the curve intersects the 50% inhibition mark on the y-axis, we can determine the concentration of the extract required for 50% inhibition of the DPPH radicals **(Belguidoum *et al., 2015*).**

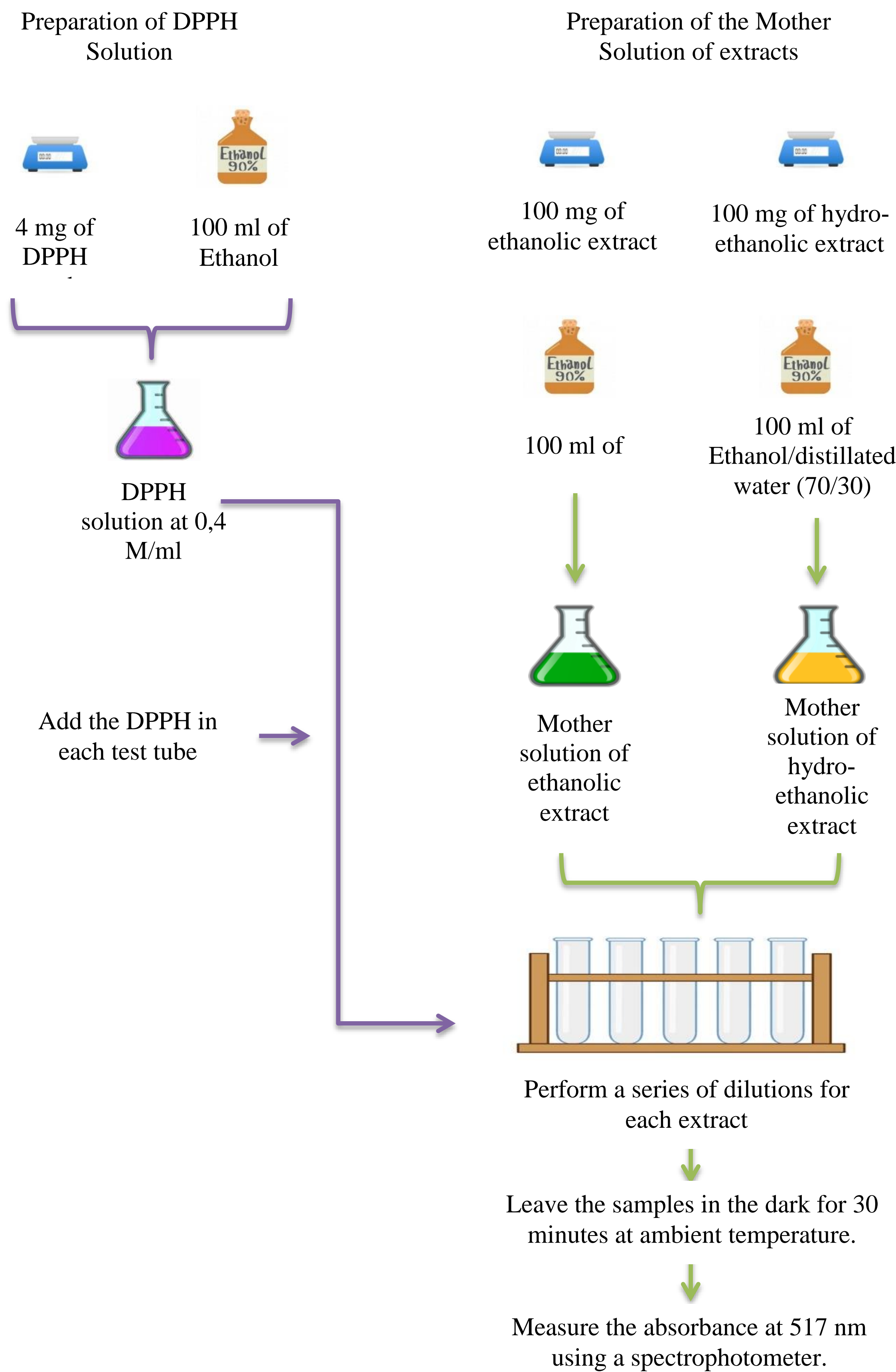

Figure 16: Protocol for evaluation of antioxidant activity

III.2. Determination of the antibacterial activity

- **The tested bacteria**

The reference bacteria necessary for this study were obtained from the biotechnology research center CRBT -CONSTANTINE- and provided by Mrs Tir Raja.

The reference bacteria used in this study are presented in table 14.

Table 14: Reference bacteria strains used in the study.

Reference bacteria	ATCC code	Gram
Escherichia coli	25922	-
Salmonella enteritidis	13076	-
Salmonella typhi	94028	-
Staphylococcus aureus	25923	+
Klebsiella pneumoniae	700603	-
Bacillus subtilis	6633	+
Bacillus cereus	10876	+
Pseudomonas aeruginosa	27853	-

III.2.1.Preparation of bacterial cultures

Preparation of a pure culture for 24 hours

- **Strain revival**

We inoculated 5 ml of nutrient broth from the stored bacterial strains. These were incubated at a temperature of 37°C for 24 hours. This step contributes to the enrichment and revitalization of the strains.

- **Subculturing of strains**

The different bacterial strains were subcultured using the streaking method to obtain isolated colonies, and then incubated at 37°C for 24 hours. This step allowed for the purification of the bacterial strains.

- **Normalization of microorganisms**

The activity of any antimicrobial agent depends on the cell density of the target strain used, hence the need to standardize the bacterial inoculum.

Each pure culture was suspended in a tube containing 5 ml of physiological serum, and the optical density of the suspensions was adjusted to 0.08 to 0.1 at 620 nm, which corresponds to a cell density close to that of 0.5 McFarland. This resulted in estimated inocula of 10^6 to 10^8 colony-forming units per milliliter (CFU/ml) **(Mala *et al., 2009*).**

- **The antibacterial activities of *Spirulina* extracts**

To evaluate the antimicrobial activity of *Spirulina* extracts (ethanolic and hydro-ethanolic), we used the agar diffusion method with the disc technique.

➢ **Principle of the disk diffusion method**

The aromatogram is based on a technique used in medical bacteriology, called the antibiogram or disk diffusion method, which is also known as the agar diffusion method. This method has the advantage of being applicable to a wide range of bacterial species and has been extensively evaluated over 50 years of global use **(Pibiri, 2006).**

The aromatogram refers to the diffusion of an antimicrobial agent of specific concentration from disks into the solid culture medium that has been inoculated. The method is based on determining a zone of inhibition proportional to the bacterial sensitivity to the antimicrobial agent present in the disk.

The discs should be distributed in a way that the zones of inhibition around the discs do not overlap, allowing the zone of inhibition to be determined. The disc diffusion method is easy to implement, reproducible, and does not require expensive equipment **(OIE Terrestrial Manual, 2008).**

- **Preparation of *Spirulina* extracts of different concentrations**

To test the antibacterial activity of the extracts (ethanolic and hydro-ethanolic), they were mixed with dimethyl sulfoxide (DMSO) and adjusted to concentrations of 0.1, 1, 10, and 100 mg/ml.

III.2.2.Antibacterial activity by disc diffusion method

- **Preparation of *Spirulina*-soaked discs**

Under aseptic conditions and using sterile forceps, sterile Wattman paper discs of 6 mm in diameter (sterilized at 120°C for 20 min by autoclaving) were impregnated with *Spirulina* extracts of different concentrations (0.1, 1, 10, and 100 mg/ml) at a rate of 50 µl per disc **(Hellal, 2011).**

- **Disk application and reading**

Under aseptic conditions, 20 ml of molten Mueller-Hinton agar medium were poured into sterile Petri dishes and allowed to solidify on the bench. The Petri dishes containing Mueller-Hinton agar were separately flooded with 1 ml of a bacterial suspension.

Using a sterile forceps sterile 6 mm diameter Wattman paper discs were placed on the surface of the Mueller-Hinton agar (5 discs per dish). The dishes were then left on the bench for 30 minutes to allow the compound to diffuse properly. Discs soaked in DMSO are used as negative controls.

The dishes were incubated at 37°C for 18 to 24 hours. (Fig. 17).

The antibacterial activity of the tested *Spirulina* extract is determined by measuring the diameters (D) of inhibition zones obtained in contact and around the discs. According to **(Ponce *et al.,* 2003)** the reading of the results is as follow:

Resistant strain ($D < 8$ mm).

Sensitive strain (9 mm $\leq D \leq$ 14 mm).

Very sensitive strain (15 mm $\leq D \leq$ 19 mm).

Extremely sensitive strain ($D > 20$ mm).

(Mala *et al., 2009*; Trabelsi *et al., 2010*; Ahsan *et al., 2015*; Ali and Doumandji, 2017; El-Monem *et al., 2018*).

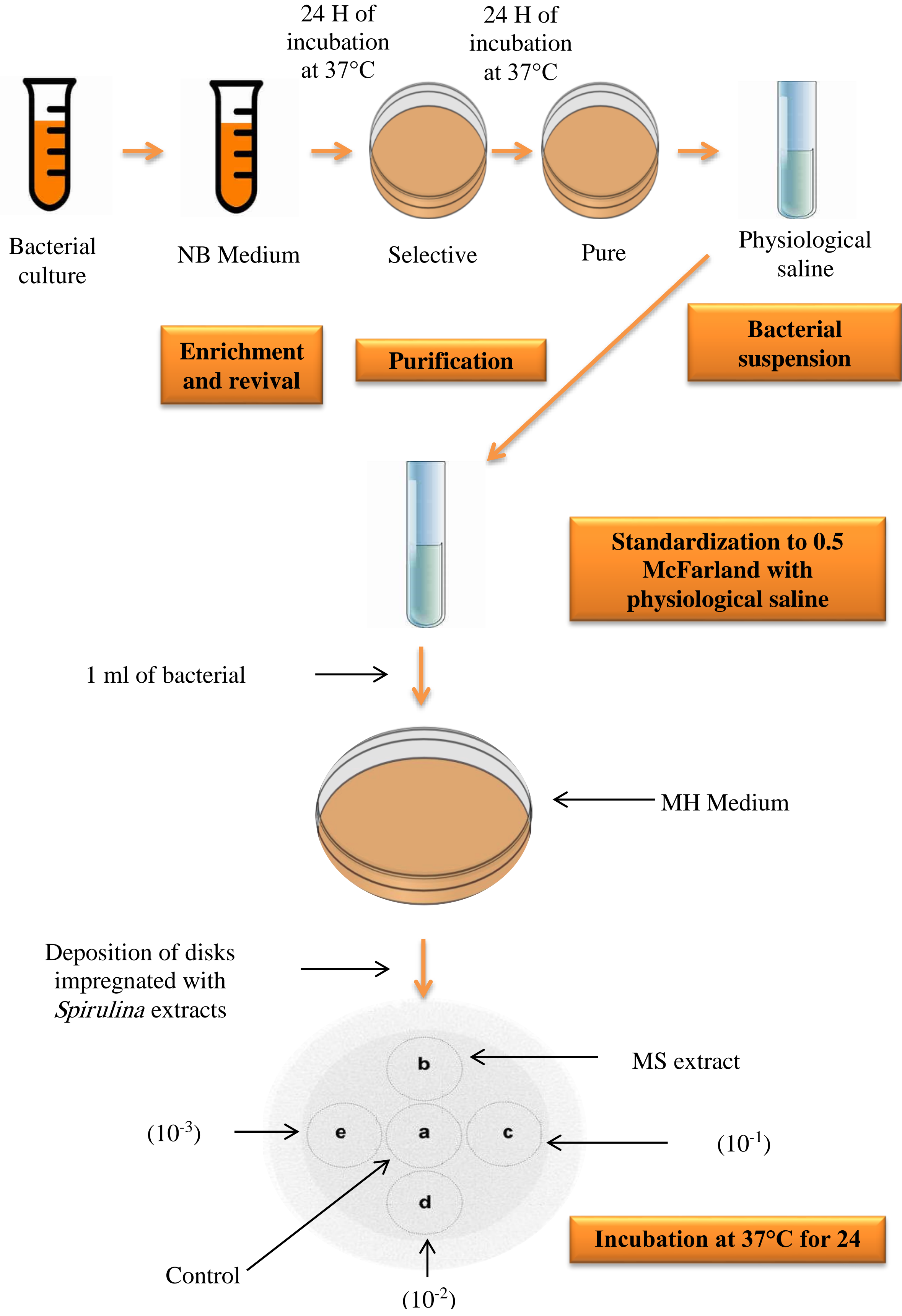

Figure 17: The antibacterial activity of *Spirulina* extracts using the disc diffusion method.

CHAPTER II. RESULTS AND DISCUSSION

II.1 Microscopic observation of *Spirulina*

From a morphological point of view, microscopic examination is a direct means of revealing the actual state of the culture. Observation in wet mount allows for the detection of contamination.

Wet mount examination

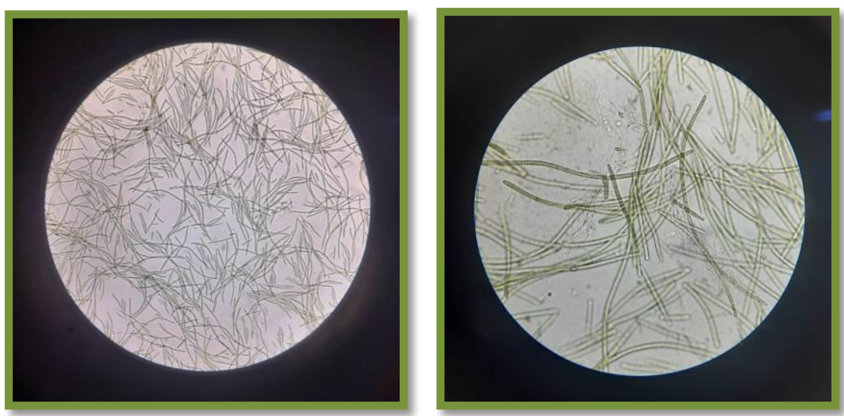

Figure 18: Microscopic observation of *S. platensis* at 10X and 40X magnification.

Microscopic observation of *Spirulina* shows that this strain is mobile and presented in a straight and filamentous form with a trichom morphology and a bright green color (figure 18). This confirms that the *Spirulina* sample was in good culture condition according to **(Muhling., 2000).**

Gram staining

Figure 19: Microscopic observation of *Spirulina* with 100X objective lens after Gram staining.

Observation under the optical microscope of the stained smear shows that *Spirulina* is pink in color, confirming that this microalgae belongs to the Gram-negative bacteria group. According to **Habib *et al*. (2008)** and **Koru (2009),** the wall of *Spirulina*, like Gram-negative bacteria, is made up of glucosamine and muramic acid associated with peptides. These carbohydrates are primarily composed of glucose, as well as rhamnose, mannose, xylose, galactose, and two unusual sugars, namely 2-O-methyl-L-rhamnose and 3-O-methyl-L-rhamnose.

II.2 Yield of extracts obtained

The maceration of 10g of *Spirulina platensis* powder in ethanol and hydro-ethanolic solution allowed for the obtainment of dry extracts with a dark green color after being dried with a rotary evaporator.

The yield of the extracts was determined in relation to the dry plant material. The results of the calculation of mass yield and percentage obtained are reported in table 15:

Table 15: Extraction yield from *Spirulina platensis* strain.

Extraction yields	Mass yield	Percentage obtained
Ethanolic solution	0,7 g	8 %
Hydro-ethanolic solution	0,9 g	9 %

Description

The yield of the hydro-ethanolic extract of *Spirulina platensis* obtained in this study is 9%, which is higher than the 7% yield obtained from the ethanolic extract.

Discussion

The higher yield of the hydro-ethanolic extract of *Spirulina platensis* compared to the ethanolic extract is due to the solvent's composition. The presence of water in the hydro-ethanolic mixture enhances the extraction of both polar and non-polar compounds, increases solubility of certain bioactive compounds, creates a synergistic effect, and helps in swelling the cell walls, making it easier for ethanol to penetrate and extract intracellular compounds. These factors collectively contribute to the higher yield.

II.3 Content of chlorophyll a, b and carotenoids

The amounts of chlorophylls and carotenoids observed in this study are shown in the Figure below:

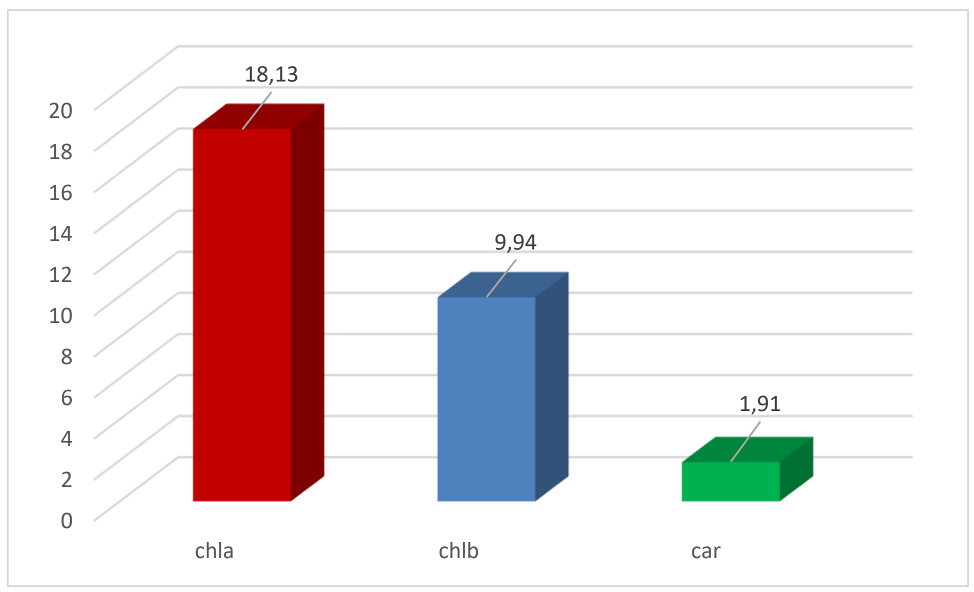

Figure 20: Content of chlorophyll a, b and carotenoids in (mg/g)

Description

Spirulina is known to provide the pure form of chlorophyll at the lowest production costs as most of the chlorophyll (about 90 %) present in *Spirulina* is in the form of chlorophyll a in particular. Chlorophyll a has recently been in high demand because its biological activity, such as that of an antioxidant, is considered to be higher than that of other chlorophylls, such as chlorophyll b. The pigments values are as follows: chlorophyll a (18.31mg/g), chlorophyll b (9,94mg/g), and carotenoids (1,91mg/g).

Discussion:

Chlorophyll a (Chl a) is the main photosynthetic pigment in microalgae compared to chlorophyll b (Chl b) **(Richmond, 2004).** The latter appears as an accessory pigment in the light-harvesting systems of green algae, constituting 30% of the total chlorophyll **(Folly, 2000).** Biomass production processes and pigment content are affected by the temperature of the culture medium. This is consistent with the study by **(Lamela *et al.,2000*),** which showed that the growth rate is directly related to the amount of chlorophyll produced.

The optimal growth temperature ranges from 20 to 40°C for *Spirulina*, depending on the strain **(Kumar *et al., 2011*).** In our study, we found the highest recorded production of chlorophyll a compared to chlorophyll b, with a low percentage of carotenoids.

Similarly, **(Externe, 2016)** found that the percentage of chlorophyll a was higher than that of chlorophyll b, which is consistent with our findings.

II.4 Extraction of phycocyanin

The concentrations and degrees of purity of phycocyanin obtained from the *Spirulina platensis* strain using the two extraction methods are represented in Figure 21:

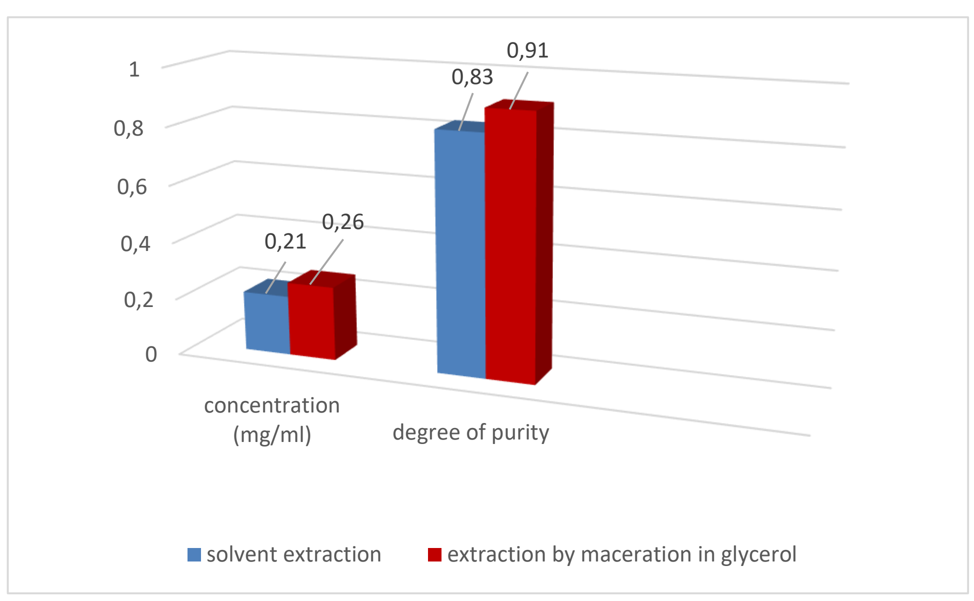

Figure 21: Concentrations and degrees of purity of phycocyanin obtained by two methods of extraction

Description

The figure presents a comparative analysis of two phycocyanin extraction methods from *Spirulina*, following the protocol outlined by **Lafri (2017).** The figure illustrates the concentration (mg/ml) and degree of purity of phycocyanin obtained through solvent extraction and maceration in glycerol. The solvent extraction method yielded a phycocyanin concentration of 0.21 mg/ml and a purity level of 0.83. In contrast, the maceration method resulted in a slightly higher concentration of 0.26 mg/ml and a purity level of 0.91.

Discussion

According to **(Pierlovisi, 2008),** the dry weight of algae consists of 30% vegetable proteins, which include protein pigments. Among these pigments, phycocyanin is the principal pigment in *Spirulina*, representing 15 to 20% of the dry weight of the algae.

When examining concentration, we observed that maceration yields were higher at 0.26 mg/ml compared to solvent extraction's 0.21 mg/ml. This suggests that maceration may facilitate better release or preservation of phycocyanin during the process. Furthermore, an assessment of phycocyanin's degree of purity indicates that maceration achieves superior purification with a value recorded at 0.91 compared to solvent extraction's 0.83.

When comparing our results to Lafri's findings, our study shows slight improvements in the concentration of phycocyanin. In Lafri's study, the solvent extraction method yielded a concentration of 0.30 mg/ml with a purity level of 0.55, while the maceration method in glycerol resulted in a concentration of 0.62 mg/ml and a purity level of 1.37. These results are higher than ours.

The extraction concentration is not only affected by the method, but also by the cultivation mode and climatic conditions from one region to another. These results are consistent with those reported by (**Lafri** ***et al., 2017***) and (**Lamela** ***et al., 2000***)**,** which demonstrated that phycocyanin represents approximately 30% of the biomass, but its concentration in cells depends on the growth conditions and strain type.

II.5 Biological activities

II.5.1 Antioxidant activity

DPPH is an organic free radical, commonly used as a reagent to evaluate the anti-radical activity of antioxidants **(Molyneux, 2004).** In this test, ascorbic acid is used as a standard.

Figure 22 shows the evolution of free radical trapping capacity by the two *Spirulina platensis* extracts.

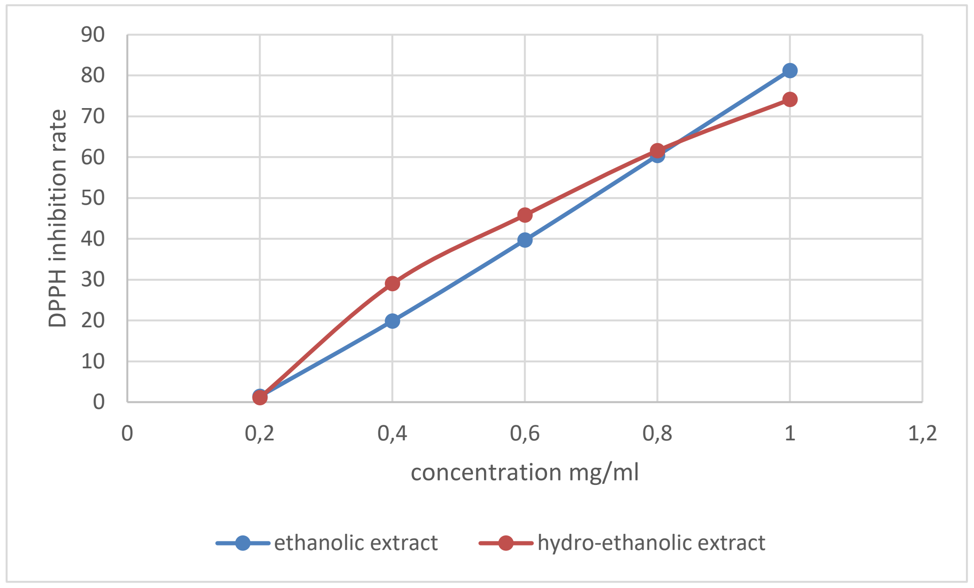

Figure 22: Percentage of DPPH inhibition as a function on different concentrations of 2 types of *Spirulina platensis* extracts

The graph illustrates the percentage of DPPH inhibition as a function of different concentrations of two types of *Spirulina platensis* extracts: ethanolic and hydro-ethanolic. The x-axis represents the concentration of the extracts, while the y-axis shows the DPPH inhibition rate. Both extracts demonstrate a positive correlation between concentration and DPPH inhibition, indicating that higher concentrations lead to increased antioxidant activity.

According to Figure 22, it can be observed that the inhibition curve of the free radical by the ethanol extract is lower than that of the hydro-ethanolic extract at all concentrations used, except for the concentration of 1 mg/ml where the ethanol extract of *Spirulina* becomes superior. This antioxidant activity of *Spirulina* is believed to be due to its richness in antioxidants **(Benmlih et Ganam., 2012).**

IC50 determination

The IC50 is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the concentration of the free radical by 50%. The lower the IC50 value, the greater the antioxidant activity of a compound **(Barkat and Laib., 2011).** The IC50 was calculated from the graph showing percentage inhibition against the concentration of the extract **(Abd-elbaky.,2009).**

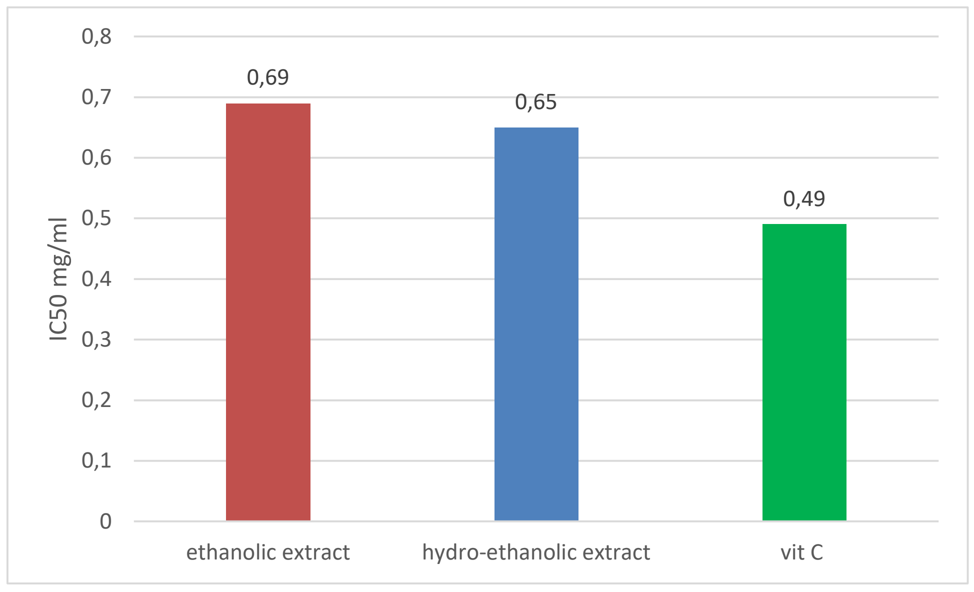

Figure 23: IC50 of different *Spirulina* extracts and ascorbic acid.

Description

According to the results obtained in Figure 23, which represent the free radical inhibitory IC50 values for the different extracts and the standard (ascorbic acid), we note that the IC50 values for the extracts are in the range of 0,69 mg/ml, 0,65 mg/ml, 0,49 mg/ml for ethanolic, hydro-ethanolic and ascorbic acid respectively.

The hydro-ethanolic extract of *Spirulina* is the most powerful antioxidant compared to the ethanolic extract.

By comparing the IC50 values of *Spirulina* extracts and ascorbic acid (Appendix A), we noticed that the IC50 of ascorbic acid is smaller than that of the extracts. Therefore, the antioxidant activity of the ascorbic acid fraction is higher than the DPPH radical scavenging capacity of the extracts. That is why we used it as a standard to compare it to our samples, where the antioxidant role of ascorbic acid derives from its reducing properties. It is the most powerful water-soluble antioxidant, capable of interacting directly with oxygen and nitrogen reactive species. This is confirmed by our results, which are consistent with **(Sekli, 2011).**

Discussion

From the results we obtained, it became clear to us that there is an inverse relationship between the percentage of free radical inhibition (IC50) and the antioxidant capacity present in the sample. This is consistent with the work of **(Pokorny *et al., 2001*).** It is in line with the work of **(Habibou *et al., 2019*)** Which means:

The inhibitory concentration (IC50) is inversely proportional to the antioxidant capacity of the compound. It expresses the amount of antioxidant required to reduce the concentration of free radicals by 50%.

The smaller the IC50 value, the greater the antioxidant activity of the compound **(Khoudali, 2014).**

The stable deep violet DPPH radical is converted to yellow DPPH after reaction with the hydrogen-donating antioxidant: DPPH. + AH→DPPH-H + A. Since the 2,2-diphenyl-1-picrylhydrazyl radical takes an electron in the presence of a free radical scavenger, the absorption decreases as a result of the color change which is stoichiometrically proportional to the number of electrons Acquired **(Zhang *et al., 2011*).**

II.5.2 Antibacterial activity

Several experimental studies have shown that *Spirulina platensis* has antimicrobial activity.

Our study results regarding the antibacterial activity produced by the cyanobacterium *S. platensis* against three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923*, Bacillus cereus* ATCC 10876*, Bacillus subtilis* ATCC 6633), and five Gram-negative bacteria (*Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, K. pneumoniae.*ATCC 700603*, Salmonella enteritidis* ATCC 13076*, Salmonella typhi* ATCC 94028) for two types of extracts (ethanolic and hydro-ethanolic) are presented in Table 16 and 17:

Table 16: Diameters of inhibition zones of ethanolic extract of *Spirulina* on eight bacterial strains.

BS \ C	MS	Dilution (10^{-1})	Dilution (10^{-2})	Dilution (10^{-3})
Bacillus cereus ATCC 10876	9 mm	9 mm	8 mm	8 mm
Pseudomonas aeruginosa ATCC 27853	13 mm	10 mm	8 mm	7 mm
Staphylococcus aureus ATCC 25923	10 mm	9 mm	9 mm	9 mm
Escherichia coli ATCC 25922	11 mm	11 mm	10 mm	9 mm
Bacillus subtilis ATCC 6633	8 mm	7 mm	7 mm	7 mm
Klebsiella pneumoniae ATCC 700603	10 mm	9 mm	7 mm	7 mm
Salmonella typhi ATCC 94028	11 mm	10 mm	10 mm	8 mm
Salmonella enteritidis ATCC 13076	10 mm	8 mm	8 mm	8 mm

Table 17: Diameters of inhibition zones of hydro-ethanolic extract of *Spirulina* on eight bacterial strains.

BS \ C	MS	Dilution (10^{-1})	Dilution (10^{-2})	Dilution (10^{-3})
Bacillus cereus ATCC 10876	6 mm	6 mm	6 mm	6 mm
Pseudomonas aeruginosa ATCC 27853	13 mm	9 mm	8 mm	7 mm
Staphylococcus aureus ATCC 25923	6 mm	6 mm	6 mm	6 mm
Escherichia coli ATCC 25922	10 mm	9 mm	7 mm	7 mm
Bacillus subtilis ATCC 6633	6 mm	6 mm	6 mm	6 mm
Klebsiella pneumoniae ATCC 700603	8 mm	7 mm	7 mm	7 mm
Salmonella typhi ATCC 94028	9 mm	6 mm	6 mm	6 mm
Salmonella enteritidis ATCC 13076	9 mm	7 mm	7 mm	7 mm

Description

The results (Table 16 and 17) of the antibacterial activity show that *Spirulina* extracts exhibit a small inhibitory activity against bacterial strains with inhibition diameters ranging from 7 mm to 13 mm. Moreover, the inhibitory effect of the ethanol extract was higher compared to the hydro-ethanolic extract.

The ethanol and hydro-ethanol extracts of *Spirulina platensis* at different concentrations showed varying ranges of inhibition zones. Generally, an increased concentration of the extract resulted in greater inhibitory activity.

Ethanolic extract

B. subtilis ATCC 6633 was resistant to all concentrations of the ethanolic extract. However *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were both sensitive to all the concentrations.

B. cereus ATCC 10876 was sensitive towards the MS and the 10^{-1} dilution of the ethanolic extract along with *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603.

S. entritidis ATCC 13076 was sensitive towards the MS only of the ethanolic extract, however *S. typhi* ATCC 94028 was sensitive towards the MS and the dilutions 10^{-1} and 10^{-2}.

Hydro-ethanolic extract

It is noticed that there is no inhibitory activity against strains Gram-positive *Bacillus cereus, Staphylococcus* aureus and *Bacillus subtilis* in the hydro-ethanolic extract. Also against Gram-negative strain *K. pneumoniae* ATCC 700603.

However, the hydro-ethanolic extracts of *Spirulina* showed a broad spectrum of activity with the highest inhibition zone of 13 mm against Gram-negative *P. aeruginosa* ATCC 27853.

Gram-negative *E.coli* ATCC 25922 was sensitive towards the MS and the first dilution 10^{-1}, while the two *Salmonella* were sensitive towards the MS only.

Discussion

According to the comparison results from this study, the tested extracts have varying antibacterial activities against the tested Gram-positive and Gram-negative microorganisms, with the ethanol extract showing the best antibacterial activity for most of the tested pathogenic strains.

The results of the antibacterial activity of *Spirulina* confirm with several previous research studies. Among these is the work of **(Chakraborty *et al.*, 2015),** which focuses on a comparative study of the antimicrobial activity of extracts from *Spirulina platensis* against Gram-positive and Gram-negative bacteria with little differences in results.

The difference in activity of the extracts against bacteria may be due to differences in physiological or biochemical properties of algae strains, or to different biotopes or culture conditions such as light, temperature, and medium **(Abedin *and Taha, 2008*).**

The extraction procedure can also lead to differences in antimicrobial activity due to variations in the nature of the solvent, the time of extraction, or the stability of antibacterial compounds in a particular solvent **(Bhuvaneswari *et al., 2013*).**

Mundt *et al*, Ozdemir *et al* have reported that the antimicrobial activity of microalgae against certain pathogenic organisms could be due to its fatty acids, hydroxylated unsaturated fatty acids, glycolipids, and phenolic compounds. The antibacterial activity of *Spirulina* can be attributed to the presence of its bioactive compounds **(Fithriani *et al.*, 2015)** such as alkaloids, flavonoids, steroids, saponins, and tannins **(Setyaningsih *et al.*,2020).** Each of these compounds has recognized antimicrobial properties, thus contributing to the overall effectiveness of *Spirulina* against various pathogens. Furthermore, phycocyanin, a blue-green pigment specific to *Spirulina*, also plays a crucial role in this antibacterial activity. Its anti-inflammatory and antioxidant properties enhance the action of the other bioactive compounds, providing a synergy that maximizes the antimicrobial effect of *Spirulina* **(Safari R *et al.*,2020).**

Furthermore, the effect of phenolic acids and fatty acids on microbial growth may result from their ability to alter the permeability of microbial cells, leading to the loss of macromolecules from the interior, and may also interact with membrane proteins causing deformation in their structure and functionality, as well as affecting cellular activity as indicated by **Mundt *et al* (2003).**

CONCLUSION

Pathogens present significant challenges within healthcare settings. More particularly the antimicrobial resistance that causes persistent infections, which necessitate targeted research.

Spirulina is a filamentous cyanobacterium. It's part from a particularly interesting bacteria called *Spirulina platensis*, known as blue-green algae. Microalgae have been the subject of many studies.

In this study, a comprehensive examination of *Spirulina* was conducted to evaluate some of its biological properties using various analytical techniques.

Microscopic observations confirmed that the *Spirulina platensis* strain is mobile and filamentous with trichome morphology, exhibiting a bright green color under the optical microscope. The pink staining in the Gram stain test indicated that *Spirulina* is Gram-negative.

Quantitative analysis revealed that *Spirulina* is a rich source of chlorophyll, particularly chlorophyll a, which constituted 18.31 mg/g of the total chlorophyll content. This high chlorophyll a content underscores *Spirulina*'s potential as a potent antioxidant. Additionally, chlorophyll b and carotenoids were present at concentrations of 9.94 mg/g and 1.91 mg/g, respectively.

Phycocyanin extraction was performed using two methods: solvent extraction and maceration in glycerol. The maceration method yielded a higher concentration (0.26 mg/ml) and purity (0.91) of phycocyanin compared to the solvent extraction method (0.21 mg/ml concentration and 0.83 purity). These results suggest that maceration in glycerol is a more efficient method for extracting high-purity phycocyanin.

The antioxidant activity was assessed using the DPPH free radical inhibition test. The IC50 values for the ethanolic and hydro-ethanolic extracts were 0.69 mg/ml and 0.65 mg/ml, respectively, compared to 0.49 mg/ml for the standard ascorbic acid. Although the IC50 value of ascorbic acid is lower, indicating higher antioxidant activity, the extracts still demonstrated significant DPPH radical scavenging capacity. Ascorbic acid's potent antioxidant role, derived from its reducing properties, serves as a benchmark for comparison.

The antibacterial activity tests demonstrated that *Spirulina* extracts exhibit inhibitory effects against various bacterial strains. The two extracts showed broad spectrum activity as highest effective zone of inhibition was recorded against *P. aeruginosa* ATCC 27853. In the same way, the ethanolic extract was active against *S. typhi* ATCC 94028 and *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 and *B.cereus* ATCC 10876 and *K.pneumniae* ATCC 700603. Moreover, the hydro-

ethanolic extract was active against *E. coli* ATCC 25922 and to a less extent against the Salmonella strains. However, B subtilis was resistant to the ethanolic extract, as well as *S. aureus* ATCC 25923 and *B.cereus* ATCC 10876 and *B. subtilis* ATCC 6633 and *K.pneumniae* ATCC 700603 which were resistant to the hydro-ethanolic extract.

In summary, the study highlights the significant antioxidant properties of *Spirulina platensis*, driven by its high content of bioactive compounds like chlorophyll a and phycocyanin, which could have valuable applications in areas like nutrition, cosmetics, and nutraceuticals. Additionally, the demonstrated antibacterial effects of *Spirulina* extracts lend credence to its traditional medicinal uses and point to possible therapeutic applications, particularly against drug-resistant pathogens. These multifaceted bioactivities highlight the versatility and promise of *Spirulina* as a natural source of medically and commercially relevant compounds.

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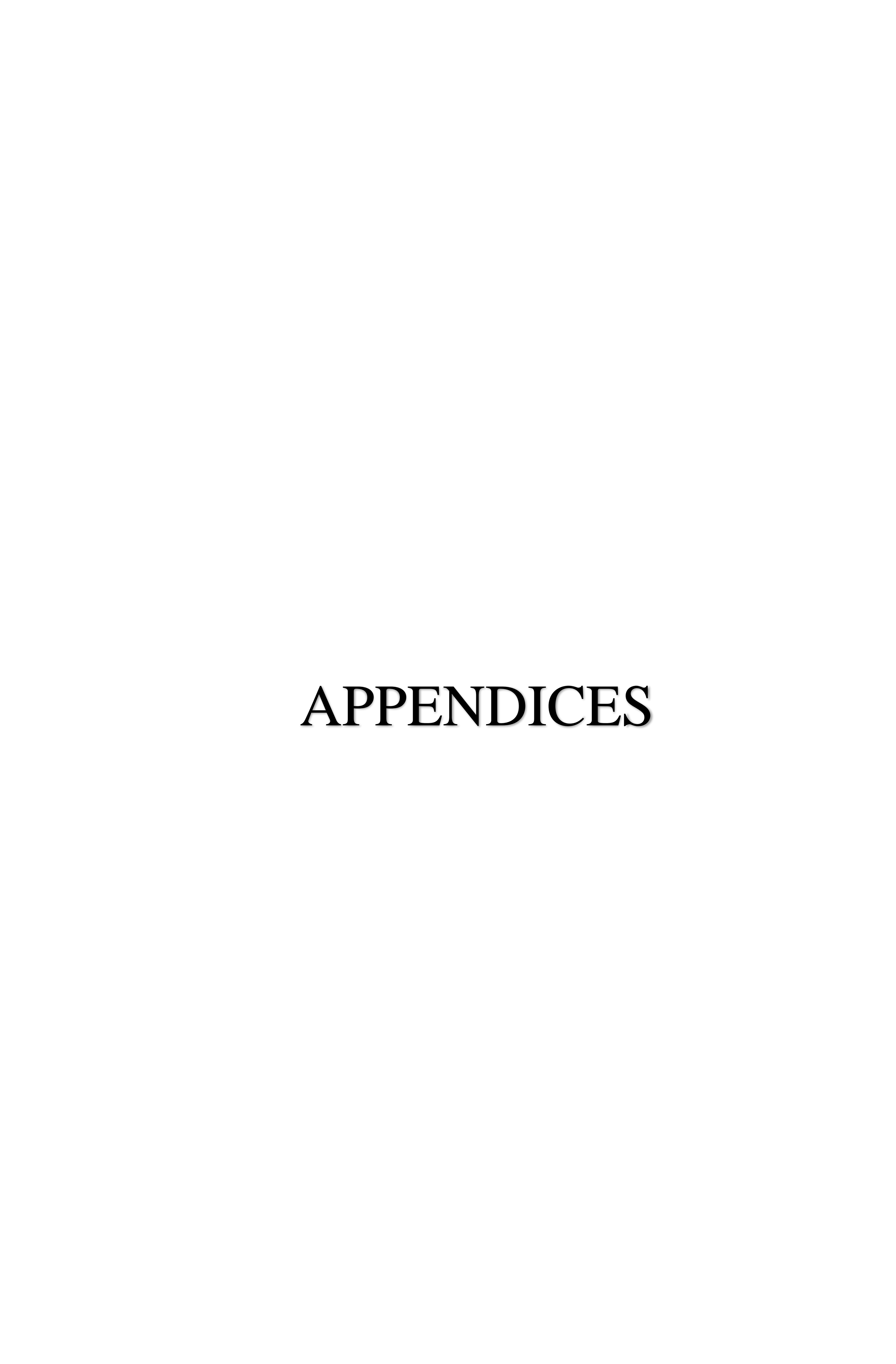
APPENDICES

APPENDIX A

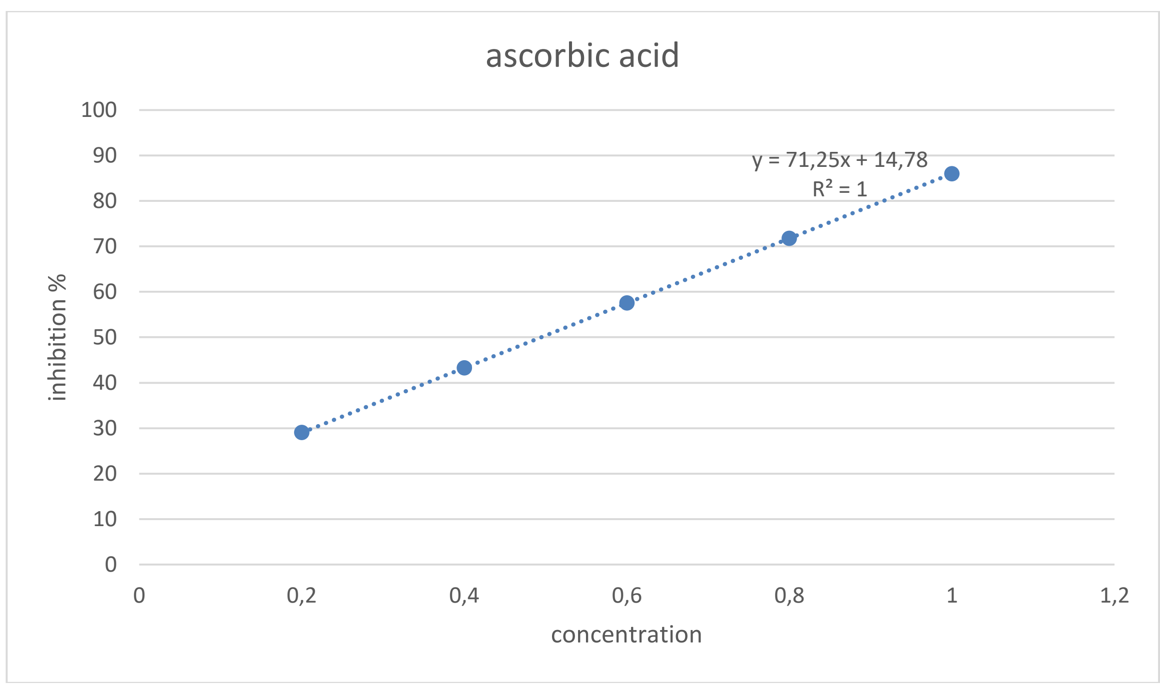

Figure 24: Vitamin C calibration curve